Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°3, 546-550
DOI: 10.24205/03276716.2020.756

Reference Interval and Medical Decision-Making Level of Anti-Thyroid Peroxidase Antibody in Pregnant Women

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Abstract

Objective: To determine the levels and positive rates of anti-thyroid peroxidase antibody (anti-TPO) in healthy non-pregnant and pregnant women, and to establish the reference interval and medical decision-making level for pregnant women in Shijiazhuang. Methods The samples of healthy non-pregnant and pregnant women in Shijiazhuang were collected, and MedCal 11.4.2.0 software was used to establish the reference interval and medical decision-making level of anti-TPO.

Results: The anti-TPO positive rates of pregnant women at different ages varied significantly. The anti-TPO positive rates of pregnant women from 23 to 30 and from 31 to 40 years old were 6% and 14%, respectively. The reference interval for pregnant women was 9.18-78.2 IU/mL. The anti-TPO medical decision-making level for thyroid dysfunction in pregnant women was 43 IU/mL at which the sensitivity was 88.75% and the specificity was 92.75%.

Conclusion: The pregnant women aged 31-40 years old have high anti-TPO positive rates. Given that the clinical decision-making values of anti-TPO kits from different manufacturers vary distinctively and there is no reference interval for pregnant women, each hospital needs to establish reference intervals and medical decision-making levels based on actual conditions.

1. Introduction

Anti-thyroid peroxidase antibody (Anti-TPO or TPO-Ab), as an autoantibody of autoimmune thyroid diseases, is a specific diagnostic index for chronic lymphocytic thyroiditis and generally used to diagnose Hashimoto's thyroiditis. In addition, anti-TPO can be used as a risk predictor for thyroid cancer (Tam A.A. et al., 2016) (Fröhlich E. et al., 2017), and an index of clinical or subclinical hypothyroidism in pregnant women. In recent years, several studies have confirmed that fetal brain development was abnormal to different extents when pregnant women suffered from mild clinical or subclinical hypothyroidism. (Moog N.K. et al., 2017) (Liu Y. et al., 2018).

At present, anti-TPO is detected mainly by double antigen sandwich immunoassay and competition assay, and the clinical decision-making values are quite different depending on manufacturers. (Tozzoli R. et al., 2017). Each manufacturer does not have a reference interval for pregnant women, so it is necessary for each medical laboratory to establish a reference interval of anti-TPO and to clarify its positive rate and medical decision-making level. The findings herein are of great clinical significance to the prevention of hypothyroidism in pregnant women and intelligence development in fetuses.

2. Materials and Methods

2.1. Subjects

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Healthy non-pregnant women: Two-hundred healthy women who received physical examinations from January to June 2019 were included. Inclusion criteria: 23-40 years old; with regular menstrual cycle; without thyroid disease, goiter in palpation, or history of hospitalization due to thyroid diseases. Exclusion criteria: Regular drinking or smoking; abuse of vitamins, oral prescription or non-prescription drugs; recent receiving of blood transfusion.

Pregnant women: A total of 194 women visiting our outpatient department from January 2019 to June 2019 were included. Inclusion criteria: 23-40 years old; without thyroid disease, goiter in palpation, or history of hospitalization due to thyroid diseases. Exclusion criteria: Regular drinking or smoking; abuse of vitamins, oral prescription or non-prescription drugs; recent receiving of blood transfusion. Seventy-one positive samples diagnosed as abnormal thyroid function by TSH test and B-scan ultrasonography were used to establish the medical decision-making level of anti-TPO.

2.2. Test method

The fasting venous blood (3 mL) of healthy non-pregnant and pregnant women was added into coagulation-promoting tube, and then centrifuged within 1 h at 3000 r/min for 10 min. The samples of hemolysis, jaundice and hemolysis were discarded. The samples were tested within 48 h using Roche electrochemiluminescence immunoassay analyzer and a matching reagent. The samples that could not be tested within 48 h were stored in a -20°C refrigerator. Afterwards, anti-TPO and Roche quality control were tested.

2.3. Outlier determination

Outliers were determined according to the D/R ratio method in the "Expert Consensus for Determining Reference Intervals and Clinical Decision-Making Levels", ranking each set of data from small to large. D refers to the absolute difference between an extreme reference value (maximum or minimum value) and an immediate extreme reference value (secondary or sub-small value), and R refers to the full distance of all reference values, i.e. the difference between maximum and minimum values. A D/R ratio of 1/3 is a critical value for excluding reference values. If D/R of a reference value exceeds one third, the extreme value should be removed. When two or three suspected outliers are on the same side of the data distribution (same for maximum or minimum values), the unique minimum outliers should be removed. If the ratio is higher than one third, all points need to be removed; if it is lower than one third, all data should be retained. If outliers are removed, the reference value should be added to a minimum of 120.

2.4. Statistical analysis

$P<0.05$ obtained by using MedCal 11.4.2.0 software was considered statistically significant. According to the gestational age, the pregnant women were divided into subpopulations, and the normality test of different subpopulations was carried out. The data were partially distributed, and subjected to the non-parametric test. The upper and lower limits of reference interval were indicated by P5 to P95, and the medical decision-making level of anti-TPO was calculated using software.

3. Results

3.1. Positive rates of subjects

The samples of 200 healthy non-pregnant women and 194 pregnant women were collected. They were divided into two groups according to their age, with 34 IUU/mL as the clinical value. The positive rates of anti-TPO for non-pregnant women aged 23-30 and 30-40 years old were 2% and 6% respectively, and those for pregnant women aged 23-30 and 31-40 years old were 6% and 14% respectively. Thus, the anti-TPO positive rate of women aged 23-30 was lower than that of women aged 31-40.

The differences between healthy non-pregnant and pregnant women in the 5th and 50th percentiles (medians) were small, but the 95th percentile of the 31-40 age group was larger than that of the 31-40 age group because of higher mean positive rate of anti-TPO. The average positive rate of anti-TPO in pregnant women was 327.22 IUU/mL and that of pregnant women was 150.50 IUU/mL, indicating that the negative rate of anti-TPO was low. Furthermore, the optimal pregnancy age was before 30, mainly because the risk of thyroid diseases increased along with aging, as evidenced by the anti-TPO positive rate of 14% in pregnant women aged 31-40 years old (Table 1).

3.2. Analysis of reference interval

Outliers were determined referring to the D/R ratio method in the "Expert Consensus for Determining Reference Intervals and Clinical Decision-Making Levels". There were three outliers for non-pregnant women and one for

pregnant women. The reference intervals of non-pregnant and pregnant women were calculated based on percentiles, with the 5th and 95th percentiles as the lower and upper limits, respectively. The reference interval of non-pregnant women (12.30-22.09 IU/mL) was different from that of pregnant women (9.18-78.2 IU/mL). In addition, the medians of anti-TPO were similar. However, the standard deviations of non-pregnant and pregnant women (23.31 vs. 41.77) were markedly different, indicating that their thyroid functions had a large difference. Since the subclinical hypothyroidism of pregnant women may affect fetal brain development, fetal intelligence is bound to be influenced in the case of delayed treatment.

Table 1: **Anti-TPO Positive Rates and Percentiles of Healthy Non-Pregnant and Pregnant Women**

Group	Age	Case number	Positive case number	P50 (P5, P95)	Mean positive rate	Positive rate
Non-pregnant women	23-30	102	2	13.84 (12.09, 20.73)	74.27	2%
	31-40	98	6	15.85 (14.24, 175.81)a	327.22	6%
Pregnant women	23-30	94	6	14.03 (8.85, 40.84)	98.35	6%
	31-40	100	14	15.58 (10.37, 115.9)b	150.50	14%

P50, P5 and P95 represent 50th, 5th and 95th percentiles, respectively. a Comparison of non-pregnant women at different ages, P<0.05; b comparison of pregnant women at different ages, P<0.05.

Table 2: **Anti-TPO Data of Healthy Non-Pregnant and Pregnant Women**

Variable	Non-pregnant women	Pregnant women
Sample size	197	193
Minimum	11.31	7.28
Maximum	233.18	328.49
Median	14.66	14.36
95% confidence interval of median	14.42-14.97	13.67-16.47
Standard deviation	23.31	41.77
Normality	P<0.0001	P<0.0001
Reference interval	12.30-22.09	9.18-78.20

Unit of anti-TPO: IU/mL

3.3. Clinical decision-making level of anti-TPO

The standardization of anti-TPO test indices remains difficult. Meanwhile, manufacturers have not determined the clinical values of thyroid dysfunction in pregnant women. Therefore, the establishment of clinical decision-making level for pregnant women with abnormal TSH test and B-scan ultrasound results, the gold standard for thyroid dysfunction, plays crucial roles in the prevention of thyroid dysfunction in pregnant women and in fetal intelligence development.

A total of 71 thyroid dysfunction samples and 193 samples diagnosed as normal thyroid function by TSH test and B-scan ultrasonography were collected to calculate the clinical decision-making level of anti-TPO in pregnant women. The diagnostic results were optimized by using 43 IUU/mL as the medical decision-making level, with a sensitivity of 88.75% and a specificity of 92.75% (Table 3 and Figure 1).

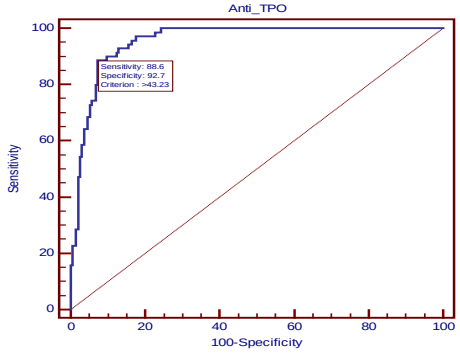

Figure 1: **ROC Curve for Anti-TPO In Pregnant Women with Abnormal Thyroid Function**

Table 3: **Medical Decision-Making Levels of Anti-TPO In Pregnant Women**

Medical decision-making level	Sensitivity	95% confidence interval	Specificity	95% confidence interval
>23.02	100	94.9-100.0	75.65	69.0-81.5
>24.58	98.57	92.3-100.0	75.65	69.0-81.5
>27.7	97.14	90.1-99.7	82.38	76.3-87.5
>28.09	94.29	86.0-98.4	83.42	77.4-88.4
>30.8	92.86	84.1-97.6	87.05	81.5-91.4
>32.82	90	80.5-95.9	87.56	82.1-91.9
>33.77	90	80.5-95.9	90.16	85.1-94.0
>36.71	88.57	78.7-94.9	90.16	85.1-94.0
>43.23*	**88.57**	**78.7-94.9**	**92.75**	**88.1-96.0**
>53.03	80	68.7-88.6	92.75	88.1-96.0
>59.43	80	68.7-88.6	93.26	88.8-96.4
>65.52	74.29	62.4-84.0	93.26	88.8-96.4

4. Discussion

TPO is the main antigenic component of thyroid microsomes, and humans themselves produce an antibody referred to as anti-TPO. Anti-TPO can indicate autoimmune response which activates complements and antibodies to exert cytotoxic effects, then causing autoimmune thyroid diseases. (Liu Y. et al., 2018) (Bhattacharyya R. et al., 2015). It has mainly been used to diagnose Hashimoto's thyroiditis, but was recently related to premature birth, abortion and hyperthyroidism or hypothyroidism. The incidence rate of abortion in anti-TPO-positive pregnant women significantly surpasses that of anti-TPO-negative ones. (Meena M. et al., 2016). Besides, both hypothyroid pregnant women and those with normal thyroid function need clinical attention. Also, early intervention should be performed for anti-TPO-positive pregnant women to prevent hypothyroidism. (Dong A.C. & Stagnaro-Green A., 2019).

According to the Roche reagent specification of 34 IUU/mL herein, the positive rates of anti-TPO in pregnant women at different ages were significantly different, being 6% and 14% in women aged 23-30 and 31-40 years old, respectively. Additionally, the positive rates of both healthy non-pregnant and pregnant women aged 31-40 were higher those of the 23-30 age groups. The mean positive rates of anti-TPO in non-pregnant and pregnant women were 327.22 and 150.50 IU/mL, respectively. Taken together, women aged under 30 are less susceptible to thyroid dysfunction.

At present, the anti-TPO detection principles and clinical decision-making values of different reagent manufacturers vary remarkably. For instance, the clinical decision-making values of anti-TPO kits developed by Roche, Siemens, Abbott and Beckman Coulter are 34, 60, 5.61 and 9 IU/mL, respectively. It has previously been reported that the positive rate of Siemens test system (85.1%) was higher than that of Roche test system (60.6%). The positive rates of different negative samples subjected to Abbott and Beckman Coulter assays were 100% and 43.5%, respectively. (Tozzoli R. et al., 2017). In addition, the manufacturers have not thoroughly studied the reference intervals of anti-TPO for non-pregnant and pregnant women hitherto.

In this study, the “Expert Consensus for Determining Reference Intervals and Clinical Decision-Making Levels” was employed as the reference. Based on the 5th and 95th percentiles, the reference interval of healthy non-pregnant women was 12.30-22.09 IUU/mL, and that of pregnant women was 9.18-87.2 IUU/mL. Moreover, we found that the anti-TPO medical decision-making level of pregnant women was 43 IUU/mL at which the sensitivity was 88.75% and the specificity was 92.75%. Nevertheless, this study still has limitations. For example, the sample size needs to be increased. Accurate reference interval and medical decision-making level can provide evidence for the diagnosis of pregnant women with abnormal thyroid function which should be relieved as early as possible to prevent harm to fetuses.

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