

NOTE

False-positive TSH receptor antibody—a pitfall of third-generation TSH receptor antibody measurements in neonates—

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Abstract. Maternal Graves' disease (GD) during pregnancy may influence thyroid function in fetuses. Neonates born to mothers with high serum TSH receptor antibody (TRAb) levels have been reported to develop 'neonatal GD'. Therefore, evaluations of serum thyroid hormone and TRAb levels in neonates upon birth are crucial for a prompt diagnosis. At delivery, we measured TRAb with third-generation TRAb test using an M22 human monoclonal antibody in neonates by collecting umbilical cord blood in a blood collection tube with lithium-heparin, which provides a whole blood/plasma sample. In recent years, we have encountered positive TRAb levels (more than 2.0 IU/L) in nineteen neonates born to mothers with GD whose thyroid hormone levels were almost within the reference range and serum TRAb levels were less than 10 IU/L. All the neonates with positive TRAb levels did not exhibit thyrotoxicosis. However, when we measured TRAb levels with serum sample in six out of the nineteen cases, their serum TRAb levels were all negative, suggesting a discrepancy of TRAb levels between in lithium-heparin plasma from umbilical cord blood and serum. Moreover, this discrepancy was observed in neonates born to euthyroid mothers, adult active GD patients and healthy volunteers. Since lithium-heparin plasma from umbilical cord blood is widely used in laboratory tests at delivery, we may encounter 'false-positive' TRAb, which may, in turn, lead to a misdiagnosis of neonatal GD. This is a pitfall of third-generation TRAb measurements in neonates, particularly at delivery, and needs to be considered by obstetricians and neonatologists.

Key words: TSH receptor antibody (TRAb), Third-generation, False-positive, Lithium-heparin plasma

MATERNAL GRAVES' DISEASE (GD) during pregnancy occurs in 0.2–0.4% of women and may influence thyroid function in fetuses [1-4]. Neonates born to these mothers especially with high serum TSH receptor antibody (TRAb) levels have been reported to develop 'neo-

natal GD'. The prevalence of neonatal GD is varying from 1.5% to 20.0% in observational cohort studies [5-9]. Newborns with neonatal GD are at risk for significant morbidity and mortality and need to be promptly identified and appropriately managed [5]. It has been reported a significant correlation between TRAb levels in cord blood in the newborns and those in their mothers at the third trimester of pregnancy and a good positive correlation between the TRAb levels in cord blood and the development of neonatal hyperthyroidism [10-12]. Therefore, evaluations of serum thyroid hormone and TRAb levels in neonates upon birth are crucial for a

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prompt diagnosis [1, 4, 5]. The current third-generation TRAb test using an M22 human monoclonal TSH receptor autoantibody conjugated with ruthenium has been widely performed [13]. In clinical practice, lithium-heparin plasma from umbilical cord blood is widely used in laboratory tests at delivery. In this manuscript, we report ‘false-positive’ TRAb in the third-generation TRAb test using lithium-heparin plasma from umbilical cord blood, which can be a pitfall of third-generation TRAb measurements in neonates, particularly at delivery.

Materials and Methods

Participants

Sixty-three women with maternal GD during pregnancy and their children who were delivered in Kyoto Medical Center from 2012 to 2016 were enrolled and retrospectively examined with their medical records. We also enrolled nine neonates born to euthyroid mothers, three adult patients with active Graves’ disease (male:female = 1:2) and four adult-healthy (euthyroid) volunteers (male:female = 2:2) as references. This study was approved by the Ethical Committees of Kyoto Medical Center (#17-031 and #17-092), and written informed consent was obtained from all patients, mothers of healthy neonates and adult-healthy volunteers.

Thyroid function tests

In all patients enrolled in the present study, serum FT₄, triiodothyronine (FT₃), and TSH levels were measured using chemiluminescence assays (ARCHITECT® FT₄, FT₃, and TSH (Abbott, Abbott Park, Illinois, U.S.A.)). Their normal ranges for adults were TSH μ U/mL, 0.5–5.0 IU/mL; FT₃, 2.30–4.30 pg/mL; and FT₄, 0.90–1.70 ng/dL. TRAb levels were also measured by chemiluminescence assays, third-generation TRAb test using an M22 human monoclonal TSH receptor autoantibody conjugated with ruthenium (Elecsys® Anti-TSHR, Roche Diagnostics Ltd., Basel, Switzerland), and a serum concentration greater than or equal to 2.0 IU/L was considered to be positive. TRAb levels of adult healthy volunteers were also measured using a two-step radioreceptor assay (DYNO test TRAb Human kit “YAMASA”; Yamasa Corp, Tokyo), which is second-generation TRAb test and the reference range is less than 1.0 IU/L. For the TRAb measurement, lithium- and/or sodium-heparin plasma and serum were employed. Lithium-heparin plasma was collected with MiniCollect® Lithium-heparin

separator tube (Greiner Bio-One, GmbH, Kremsmünster, Austria). Sodium-heparin plasma and serum were collected with vacuum blood collection tubes (VENOJECT II®, #VP-H050K, TERUMO, Tokyo, Japan and #OP-SP0507-2, NIPRO, Osaka, Japan, respectively).

Statistical analysis

Statistical analysis to evaluate correlation between maternal serum TRAb levels and those in umbilical cord blood with lithium-heparin plasma were performed by Spearman rank correlation coefficient using Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). The undetectable (UD) TRAb levels were simply assigned a ‘zero’ value.

Results

A 40-week, 3,872 g male neonate (#1 in Table 1) was born *via* vaginal delivery to a 32-year-old uniparous mother. She suffered from GD at the age of 27 and was treated with anti-thyroid drug. She had been in a complete remission since she was 28 years old. At this pregnancy, she consulted an endocrinologist and underwent thyroid function tests. Her thyroid function and serum TRAb levels (third-generation test) had been normal and negative, respectively throughout the pregnancy. Serum free T₄ levels of the neonate were 1.30 ng/dL, which was within normal range for neonates [14, 15]. However, TRAb levels in umbilical cord blood were positive (4.61 IU/L, above 2.0). Therefore, the neonate had been observed regularly on monthly basis and TRAb tests had been performed using lithium-heparin plasma as was used with the cord blood. His TRAb levels had been above 2.0 and around 3.0 IU/L thereafter, whereas his thyroid function had been within normal range [14, 15]. At the age of 2 years and 3 months, TRAb tests were performed using his serum and revealed his serum TRAb levels were negative and undetectable. This discrepancy in TRAb levels between in serum and lithium-heparin plasma prompted us to check serum TRAb levels in the neonates born to mothers with GD, who showed positive in umbilical cord blood with lithium-heparin plasma (Table 1).

Retrospectively, we found positive TRAb levels (more than 2.0 IU/L) in nineteen neonates born to mothers with GD, whose thyroid hormone levels were almost within the reference range and serum TRAb levels were less than 10 IU/L, which is the upper limit of maternal serum TRAb levels to avoid neonatal GD [9]. TRAb levels in

Table 1 Six neonatal cases, that showed discrepancy in TRAb levels between serum and umbilical cord blood in a blood collection tube with lithium-heparin.

#	Mothers					Neonates								
	TSH ($\mu\text{U/mL}$)	FT3 (pg/mL)	FT4 (ng/dL)	TRAb (IU/L)	ATD	Gestational age (week)	Birth weight (g)	Sex	TSH ($\mu\text{U/mL}$)	FT3 (pg/mL)	FT4 (ng/dL)	TRAb (lithium- heparin plasma) (IU/L)	TRAb (serum) (IU/L)	Timing of serum TRAb measurement
1	1.68	2.55	1.11	UD	-	40	3,872	M	8.32	N/A	1.30	4.61	UD	2 years and 3 months
2	N/A	N/A	N/A	UD	+	37	3,108	F	9.60	1.50	1.10	3.31	0.35	2 years and 2 months
3	1.25	2.00	0.90	3.46	-	40	2,764	F	6.27	N/A	1.30	2.76	UD	1 year and 7 months
4	0.49	2.20	1.00	0.39	-	37	2,104	M	12.07	1.70	1.20	2.84	UD	9 months
5	<0.01	5.30	2.00	5.85	+	40	3,112	M	2.45	1.30	1.40	3.11	UD	9 months
6	<0.01	4.02	1.35	0.74	+	39	4,002	M	1.34	1.65	1.17	3.46	0.58	15 days

Thyroid function and TRAb levels at the delivery are indicated for both mothers and neonates.

ATD, anti-thyroid drugs administration during pregnancy; N/A, not applicable; UD, undetectable

umbilical cord blood measured with lithium-heparin plasma were not statistically correlated with maternal serum TRAb levels (Fig. 1). All the neonates with positive TRAb levels did not exhibit thyrotoxicosis [14, 15]. Serum TRAb levels were evaluated in six out of the nineteen cases, who had visited for the neonatal follow-up (Table 1). The neonates (#1~#5) had been followed up because they showed positive TRAb several times using lithium-heparin plasma until serum TRAb levels were evaluated, which showed negative. Therefore, we evaluated serum TRAb levels on the fifteen days after birth for the neonate #6 (Table 1), who showed positive TRAb using lithium-heparin plasma from umbilical cord blood at birth.

We performed third-generation TRAb test on nine neonates born to euthyroid mothers using serum and lithium- and sodium-heparin plasma from umbilical cord blood. Although serum TRAb levels (third-generation test) were undetectable in all of the neonates, they were measurable with lithium- and sodium-heparin plasma in all of the neonates. Moreover, 3 and 2 out of 9 neonates showed positive TRAb levels (more than 2.0 IU/L) with lithium- and sodium-heparin plasma, respectively (Table 2). Of note, TRAb levels measured by second-generation test with lithium-heparin plasma were undetectable in all of the neonates who underwent the test. In addition, we conducted third-generation TRAb

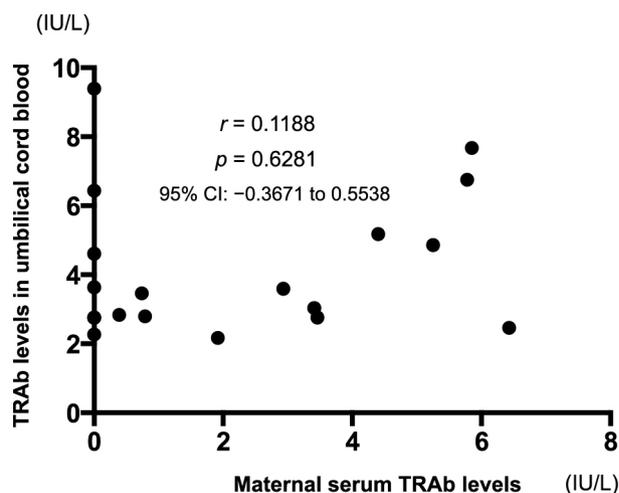


Fig. 1 Correlation between maternal serum TRAb levels and those in umbilical cord blood.

Maternal serum TRAb levels were evaluated at the third trimester of pregnancy. TRAb levels in umbilical cord were measured with using lithium-heparin plasma. CI: confidence interval

test on adult patients with active GD and adult-healthy (euthyroid) volunteers using serum and lithium- and sodium-heparin plasma. In all of the patients with active GD, TRAb levels with lithium-heparin plasma were higher than those with serum. In all but one of the

Table 2 Nine neonates born to euthyroid mothers

#	Serum				Plasma		
	TSH (μ U/mL)	FT3 (pg/mL)	FT4 (ng/dL)	TRAb (IU/L)	TRAb (lithium-heparin plasma) (IU/L)	TRAb (sodium-heparin plasma) (IU/L)	TRAb (lithium-heparin plasma) (second-generation) (IU/L)
1	4.68	UD	1.00	UD	1.39	1.16	UD
2	3.27	1.30	1.07	UD	1.15	1.03	N/A
3	6.40	1.35	1.28	UD	1.98	1.88	UD
4	7.20	1.35	1.13	UD	2.06	1.78	UD
5	6.26	1.71	1.13	UD	2.63	2.37	UD
6	3.96	1.17	1.27	UD	2.06	2.05	UD
7	22.61	1.07	1.13	UD	1.52	1.10	UD
8	5.52	1.05	1.09	UD	0.95	0.37	N/A
9	11.24	1.53	1.20	UD	1.90	1.55	N/A

Thyroid function and TRAb levels at the delivery are indicated for neonates.

N/A, not applicable; UD, undetectable

Table 3 Serum and lithium- and sodium-heparin plasma TRAb levels of adult patients with Graves' disease (#1–3) and adult-healthy volunteers (#4–7)

#	Age (years old)	Sex	TSH (μ U/mL)	FT3 (pg/mL)	FT4 (ng/dL)	TRAb (serum) (IU/L)	TRAb (lithium-heparin plasma) (IU/L)	TRAb (sodium-heparin plasma) (IU/L)	TRAb (lithium-heparin plasma) (second- generation) (IU/L)
1	22	F	UD	32.7	7.78	58.19	71.86	57.86	N/A
2	28	F	3.61	2.51	0.92	2.78	5.62	5.71	N/A
3	25	M	UD	5.64	1.88	23.46	26.69	26.93	N/A
4	32	M	0.92	3.11	1.02	UD	2.37	1.93	UD
5	26	F	1.49	3.20	1.15	UD	2.86	0.30	UD
6	40	M	1.06	2.72	0.86	UD	2.70	UD	UD
7	40	F	1.48	2.43	0.99	UD	2.77	3.06	UD

N/A, not applicable; UD, undetectable

patients, TRAb levels with sodium-heparin plasma were higher than those with serum. Moreover, in all of the adult-healthy volunteers, TRAb levels with lithium-heparin plasma were positive and in one of them, TRAb levels with sodium-heparin plasma were positive, whereas they were all negative and undetectable with serum (Table 3). TRAb levels measured by second-generation test with lithium-heparin plasma were negative (less than 1.0 IU/L) in all of the adult-healthy volunteers (Table 3).

Discussion

In the current study, in third-generation TRAb test for neonates, we experienced 'positive' and 'negative' TRAb with lithium-heparin plasma and serum, respectively, suggesting a discrepancy between the two samples. Since 'positive' TRAb was seen with lithium- and sodium-heparin plasma but not with serum in the neonates born to euthyroid mothers and adult-healthy volunteers, 'false-positive' TRAb could be potentially seen both in normal neonates and adults. Moreover, since this discrepancy was not found in second-generation TRAb

test using radioreceptor assay, ‘false-positive’ TRAb with lithium-heparin plasma may be specific to third-generation TRAb test. However, we demonstrated a relatively small number of samples and the mechanisms responsible for positive TRAb in third-generation test using plasma samples remain elusive, which may be a limitation of this report.

Given TRAb levels with lithium-heparin plasma were higher than those with serum in the patients with active GD, we speculated that some factors in lithium-heparin plasma would increase TRAb levels in third-generation TRAb test. The current third-generation TRAb test using an M22 human monoclonal TSH receptor autoantibody conjugated with ruthenium has been widely performed [13]. Up to date, a lot of reports have indicated no significant differences between serum and lithium-heparin matched samples in electrochemiluminescence immunoassays including Elecsys® kit, which was employed for third-generation TRAb test in the current study [16-18]. On the other hand, false-positive values for Troponin I and Troponin T assays in lithium-heparin plasma have been reported, which may be attributed to residual fibrin in plasma [19-23]. Therefore, it may be related to false-positive TRAb levels. In addition, hemolysis has been reported to affect electrochemiluminescence immunoassays, which are used in third-generation

TRAb test [24-26]. Thus, false-positive TRAb levels may also be attributed to cell-free hemoglobin in plasma, even though severe hemolysis was not found in the plasma samples used in the current report. On the other hand, since ‘false-positive’ TRAb was found both with lithium- and sodium-heparin plasma in the neonatal and adult volunteers (Tables 2, 3), heparin plasma *per se*, may affect the phenomenon.

Since lithium-heparin plasma from umbilical cord blood is widely used in laboratory tests at delivery, we may encounter ‘false-positive’ TRAb, which may, in turn, lead to a misdiagnosis of neonatal Graves’ disease. This is a pitfall of third-generation TRAb measurements in neonates, particularly at delivery, and needs to be considered by obstetricians and neonatologists.

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