

BRIEF COMMUNICATION

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A Retrospective Study on the Association between Thyroid Autoantibodies with β 2-glycoprotein and Cardiolipin Antibodies in Recurrent Miscarriage

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ABSTRACT

Etiologic factors for recurrent miscarriage (RM) include autoimmune diseases, the most frequently antiphospholipid syndrome and thyroiditis. Some women who suffer from RM might also have an altered immune system. We aimed to evaluate possible associations between anti-thyroid and anti-phospholipid antibodies in women with RM.

In a retrospective case series 1 on 156 women with RM, major outcome parameters were antibodies against cardiolipin, β 2-glycoprotein I, thyreoperoxidase (TPO-Ab), and thyroglobulin (TG-Ab).

Significant ($p < 0.05$) positive correlations were found between TPO-Ab and TG-Ab ($r = 0.577$), TPO-Ab and IgG anti-cardiolipin antibodies ($r = 0.284$), TPO-Ab and IgG anti- β 2-glycoprotein I antibodies ($r = 0.196$), and TG-Ab and IgG anti-cardiolipin antibodies ($r = 0.193$), as well as between all types of anti-phospholipid antibodies. Women with both increased TPO-Ab and TG-Ab levels revealed higher ($p < 0.001$) IgG anti-cardiolipin and IgG anti- β 2-glycoprotein I antibodies.

Anti-thyroid antibodies were linked to anti-phospholipid antibodies and should be in the focus of future research on RM.

Keywords: Antiphospholipid syndrome; Autoimmunity; Dehydroepiandrosterone; Recurrent early pregnancy loss; Recurrent miscarriage; Thyroiditis

INTRODUCTION

Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy losses, with the same

partner, before the completed 20th week of gestation.¹ General etiological categories of RM include genetic, endocrine, anatomical, immunological, thrombophilic, and environmental factors.² In recent years, scientific interest has focused on the maternal immune system as it undergoes specific changes during pregnancy. These changes enable the maternal toleration of the semi-allogeneic conceptus.³ In the diagnostic evaluation of RM, one of the major contributory immune factors is

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antiphospholipid syndrome, which can be found in 5–15% of cases.² In addition, thyroid autoimmunity has been demonstrated as an independent risk factor for RM. According to a recent meta-analysis, women with RM more often demonstrated increased levels of thyroid antibodies than controls. Thus, thyroid autoimmunity seems to influence independent of secondary hypothyroidism.⁴

Autoimmune thyroiditis, especially Hashimoto's thyroiditis, is linked with other autoimmune diseases and this is likely derived from a polyclonal autoimmune response against organ-specific autoantigens.⁵ The association between thyroid autoimmunity and antiphospholipid syndrome has been known for a long time.^{6,7} However, it could not be proven in women with RM⁸ which might have been due to the low sample size. The association could be of special impact for women who suffer from RM, since the easily screenable and detectable anti-thyroid antibodies might only represent the "tip of an autoimmune iceberg", at least in some affected patients. Thus, we aimed to retrospectively evaluate possible associations between anti-thyroid antibodies and anti-cardiolipin/anti- β 2-glycoprotein antibodies in women with RM. As a secondary objective, we also focused on dehydroepiandrosterone-sulfates (DHEAS), since it have been reported to be associated with autoimmune diseases,⁹ including Hashimoto's thyroiditis.¹⁰

PATIENTS AND METHODS

All 156 women with RM who had undergone a complete diagnostic evaluation at our department between January 2003 and January 2013 were included in this retrospective study. RM was defined based on documented history of at least three spontaneous, consecutive miscarriages before 15th weeks of gestation, with the same partner. We primarily hypothesized that levels of anti-thyroid antibodies would correlate with levels of antibodies suggestive of antiphospholipid syndrome. The study was approved by the Institutional Review Board of the Medical University of Vienna (IRB number 1098/2013). All scientific work conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

All data were retrieved by retrospective chart review. Basic patient characteristics included age, body

mass index (BMI), and the number of previous first trimester miscarriages. All women had underwent the following standard diagnostic evaluation: diagnostic hysteroscopy; thrombophilia screening, including protein S antigen, protein C activity, aPC-resistance, and antithrombin III activity; paternal and maternal karyotype; cervical cultures for chlamydia, ureaplasma, and mycoplasma; a comprehensive hormonal status, including TSH and antibodies against thyroperoxidase (TPO-Ab; normal range <34 IU/mL) and thyroglobulin (TG-Ab; normal range <33 IU/mL); and evaluation of antiphospholipid syndrome with IgM and IgG anti-cardiolipin antibody assessment (normal ranges: <7 U/mL and <10 U/mL, respectively) and IgM and IgG anti- β 2-glycoprotein I antibody assessment (normal ranges of <8 U/mL)¹¹ according to the revised Sydney criteria.¹² Moreover, serum levels of thyroid stimulating hormone (TSH), prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, androstenedione, DHEAS, and 17-hydroxy-progesterone on days 3-5 of a normal menstrual cycle had been also measured, and a vaginal ultrasound had been performed.

Nominal variables are reported as numbers and frequencies, and continuous variables as median and interquartile range (IQR). Statistical analyses were performed with the SPSS software package, version 19 (SPSS, Chicago, USA). Differences between groups were tested using the Fisher's exact test for nominal variables and analysis of variance (ANOVA) or Welsch test for numeric variables. Correlations were calculated using the Pearson test. Differences were considered statistically significant if $p < 0.05$.

RESULTS

Patients' median age was 33 years at the diagnostic evaluation for RM (IQR, 28-37). Median BMI was 25.3 kg/m² (IQR, 21.4-30.0). Women had suffered from three, four or more than five previous first trimester miscarriages in 113 (72.4%), 31 (19.9%), and 12 (7.7%) cases, respectively. Median serum levels of autoantibody testing and DHEAS are summarized in Table 1.

Table 2 shows the details of correlation analyses. Significant positive correlations were found between TPO-Ab and TG-Ab ($r=0.577$, $p < 0.001$), TPO-Ab and IgG anti-cardiolipin antibodies ($r=0.284$, $p < 0.001$), TPO-Ab and IgG anti- β 2-glycoprotein I antibodies

Table 1. Median serum levels of autoantibodies and DHEAS in women with recurrent miscarriage

TPO-Ab (IU/mL)	8 (5;12)
TG-Ab (IU/mL)	12 (10;18)
IgG anti-cardiolipin antibodies (U/mL)	2.0 (1.0;3.6)
IgM anti-cardiolipin antibodies (U/mL)	1.8 (1.1;2.8)
IgG anti-β2-glycoproteinI antibodies (U/mL)	0.5 (0.5;2-1)
IgM anti-β2-glycoproteinI antibodies (U/mL)	1.2 (0.7;2.1)
DHEAS (μg/mL)	1.7 (1.2;2.3)

Data are presented as median and interquartile ranges

($r=0.196$, $p=0.007$), and TG-Ab and IgG anti-cardiolipin antibodies ($r=0.193$, $p=0.008$). Furthermore, all types of anti-cardiolipin and anti-β2-glycoprotein I antibodies were positively correlated with each other in a highly significant manner ($p<0.001$). A significant negative correlation was found between DHEAS and TPO-Ab ($r=-0.179$, $p=0.013$). Notably, none of the parameters were correlated with the number of pregnancy losses ($p>0.05$; data not shown).

Twenty-nine women (18.6%) had either TPO-Ab or TG-Ab levels that exceeded the upper normal range. In eight patients (5.1%), there was one increased marker for antiphospholipid syndrome. In women with elevated anti-thyroid antibodies, 13.8% (4/29) had one increased marker for antiphospholipid syndrome,

compared to 2.4% in women with normal TPO-Ab and TG-Ab levels (4/127; $p=0.040$).

When comparing women with both increased TPO-Ab and TG-Ab levels ($n=12$) to the remaining 144 women, the former revealed significantly higher levels 1.6-8.6, vs. 1.9U/mL, IQR 1.0-3.3, respectively; $p<0.001$) and IgG anti-β2-glycoprotein I antibodies (2.9 U/mL, IQR 0.7-5.6, vs. 0.5 U/mL, IQR 0.5-2.0, respectively; $p<0.001$).

There was neither significant difference in IgM anti-cardiolipin antibodies (2.5 U/mL, IQR 2.0-4.6, vs. 1.8 U/mL, IQR 1.0-2.8, respectively; $p=0.060$) nor in IgM anti-β2-glycoprotein I antibodies (2.1 U/mL, IQR 0.7-3.1, vs. 1.1 U/mL, 0.7-2.0, respectively; $p=0.096$). There were no significant

Table 2. Correlations of serum antibody and DHEAS levels in women with recurrent miscarriage

		TPO-Ab	TG-Ab	IgG anti-cardiolipin antibodies	IgM anti-cardiolipin antibodies	IgG anti-β2-glycoprotein I antibodies	IgM anti-β2-glycoprotein I antibodies	DHEAS
TPO-Ab	r	-	0.577	0.284	0.080	0.196	0.094	-0.179
	p	-	<0.001	<0.001	0.159	0.007	0.123	0.013
TG-Ab	r	0.577	-	0.193	0.002	0.087	-0.045	-0.128
	p	<0.001	-	0.008	0.489	0.140	0.289	0.056
IgG anti-cardiolipin antibodies	r	0.284	0.193	-	0.576	0.570	0.411	-0.023
	p	<0.001	0.008	-	<0.001	<0.001	<0.001	0.389
IgM anti-cardiolipin antibodies	r	0.080	0.002	0.576	-	0.545	0.721	-0.002
	p	0.159	0.489	0.000	-	<0.001	<0.001	0.489
IgG anti-β2-glycoproteinI antibodies	r	0.196	0.087	0.570	0.545	-	0.469	-0.031
	p	0.007	0.140	<0.001	<0.001	-	<0.001	0.350
IgM anti-β2-glycoprotein I antibodies	r	0.094	-0.045	0.411	0.721	0.469	-	0.046
	p	0.123	0.289	<0.001	<0.001	<0.001	-	0.286
DHEAS	r	-0.179	-0.128	-0.023	-0.002	-0.031	0.046	-
	p	0.013	0.056	0.389	0.489	0.350	0.286	-

*Correlations calculated by the Pearson test

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differences in markers for antiphospholipid syndrome between women who revealed either increased TPO-Ab or TG-Ab levels (n=29) to those without serologic signs of autoimmunity (n=127; data not shown in detail).

DISCUSSION

This retrospective case series revealed the following main results: in women with RM, levels of anti-thyroid antibodies were positively correlated with IgG antibodies suggestive of antiphospholipid syndrome; DHEAS was negatively correlated with TPO-Ab; and women positive for both anti-thyroid antibodies revealed significantly higher IgG anti-cardiolipin antibody and IgG anti- β 2-glycoprotein I antibody levels, which was not the case for women with only one increased anti-thyroid antibody.

These results highlight the importance of anti-thyroid antibodies. They could represent an easily detectable sign of an altered immune system. It has already been mentioned that autoimmune thyroiditis is, indeed, associated with a variety of other autoimmune diseases.^{7,13} These include both organ-specific and non-organ-specific autoimmune diseases.¹³ Notably, autoimmune coagulation disorders have been reported to be associated with Hashimoto's thyroiditis, i.e., autoimmune thrombocytopenic purpura, antiphospholipid syndrome, and autoantibodies against coagulation factor VIII.¹⁴ This could be of special relevance in the case of RM, keeping in mind that thrombophilic factors play a major etiologic role.

Another interesting fact about TPO-Ab levels is their negative correlation with DHEAS. This has been reported previously,^{10,15} and it has been suggested that DHEA possibly lowers TPO-Ab.¹⁰ This seems reasonable, since T-helper cell type 1 responses predominate in Hashimoto's thyroiditis, and DHEA can reduce the release of type 1 inflammatory cytokines. In addition, it exerts other complex interactions between androgens and cytokines.¹⁶ In contrast; antiphospholipid syndrome is an autoimmune disease with a dominant type 2 cytokine response. Accordingly, none of the parameters suggestive of antiphospholipid syndrome was correlated with DHEAS levels. These findings; however, could disprove the general importance of DHEA/DHEAS in autoimmunity, at least in our patient population.

Notably, none of the parameters for autoimmunity was correlated with the number of previous pregnancy losses. This seems somewhat in contrast to previous reports about a positive correlation between TPO-Ab levels and previous pregnancies.^{17,18} Fetal microchimerism has been blamed for this association. However, the latter was true for parity, but not for gravidity. It seems reasonable that there might be a different mechanism in women with RM: the development of autoimmunity might not be based on pregnancy, but the reverse could be the case with autoimmunity being a major etiologic factor for pregnancy losses. Thus, the number of RM could not be linked with the presence of an autoimmune disease like Hashimoto's thyroiditis.

When discussing "the chicken or the egg" causality dilemmas, the association between autoimmune thyroiditis and RM could also be an important focus.⁴ Usually, type 1 autoimmune diseases, such as Hashimoto's thyroiditis, would improve during a pregnancy through antibody declines, which are due to a switch to a predominantly T-helper-2-type pattern of cytokines.¹⁹ But, how could this autoimmune disease cause miscarriage on an immunologic basis? Hypothetically speaking, an altered overall immune situation that favors type 1 cytokine responses could impair the switch to the T-helper-2-type pattern necessary to induce maternal immune tolerance, and, thus, maintain the pregnancy. This assumption leads us back to the necessity of evaluating the immune system of women with RM in more detail, probably also during a pregnancy. We do not assume that all women with serologic signs of autoimmune thyroiditis suffer from a generally altered immune system, but some could. Evaluating such a phenomenon should be our future research aim.

Our study must be interpreted with caution due to its retrospective design and the small sample size. The fact that all types of anti-phospholipid antibodies were positively correlated with each other, as well as the fact that TPO-Ab and TG-Ab were correlated with each other (Table 2), supports the reliability of the data set. Of course, we were able to show only associations and no cause-and effect relations.

In conclusion, the role of thyroid autoimmunity should be the focus of future prospective studies on RM, considering the association with other important autoimmune diseases.

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