

Prevalence of anticardiolipin antibodies in type 1 diabetes and autoimmune thyroiditis

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KEY WORDS

anticardiolipin antibodies, autoimmune thyroiditis, diabetes mellitus

ABSTRACT

INTRODUCTION High levels of anticardiolipin (aCL) antibodies may predict vascular complications that could develop in type 1 diabetes and autoimmune thyroiditis (AIT). However, the clinical relevance of these antibodies in subjects with type 1 diabetes and AIT is unclear.

OBJECTIVES The aim of the study was to determine the prevalence and significance of aCL antibodies in patients with type 1 diabetes and AIT.

PATIENTS AND METHODS The study involved 74 patients with type 1 diabetes (mean age 12.9 ± 4.2 years), 64 patients with AIT (mean age 14.1 ± 3.7 years), and 35 healthy control subjects (mean age 12.8 ± 3.3 years). The levels of aCL immunoglobulin (Ig) G and aCL IgM antibodies were measured by enzyme-linked immunosorbent assays. Low-positive and medium/high-positive cut-off values were selected for aCL antibody positivity.

RESULTS The prevalence of aCL antibodies was higher in AIT patients compared with diabetic and healthy subjects with low positive levels ($P < 0.05$), while the frequency of medium/high aCL positive levels in AIT and diabetic subjects was not statistically different from that observed in healthy subjects.

CONCLUSIONS Our study showed an increased prevalence of aCL antibody positivity in patients with AIT at a low-positive aCL cut-off level, while the frequency of aCL antibody positivity at a moderate/high aCL cut-off level was not significantly different between the groups. We believe that routine investigation of aCL levels may not have clinical relevance in children with type 1 diabetes or AIT.

INTRODUCTION Antiphospholipid syndrome (APS) is associated with the production of anticardiolipin (aCL) antibodies, which develop against the negatively charged phospholipids present in cell membranes.^{1,2} The syndrome may coexist with several, predominantly autoimmune diseases (secondary APS), or it may be present without any other disease (primary APS).² It has been reported that the incidence of aCL antibody positivity increases with age and concomitant chronic diseases.^{3,4} However, the exact mechanism by which antiphospholipid antibodies induce the thrombophilic state remains unknown.²

Type 1 diabetes and autoimmune thyroiditis (AIT) are indicators of immune dysregulation that develops as a result of an autoimmune process. In addition, an increasing number of other autoantibodies and abnormalities of cell-mediated

immunity has been observed in many subjects with type 1 diabetes and AIT. It has been reported that patients with type 1 diabetes and AIT have high levels of aCL antibodies as a marker of autoimmune dysregulation.⁵⁻⁸ It has also been suggested that high levels of aCL antibodies might predict vascular complications in diabetic patients.^{1,9,10} Anzai et al.¹¹ reported the presence of aCL antibodies in AKR/J mice with streptozocin-induced diabetes. Subsequently, a few more studies have been conducted to show the positivity of aCL antibodies in children with type 1 diabetes. However, there have been no studies evaluating aCL antibody positivity in children with AIT so far.^{1,5,7-9,12}

A few studies provided conflicting results on the prevalence of aCL antibodies in adult subjects with AIT.^{7,8,12} In 1994, Paggi et al.⁸

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reported a study in which 4 out of 5 subjects with Hashimoto's thyroiditis showed the presence of aCL antibodies. Diez et al.¹² found no significant difference in the prevalence of aCL antibodies between the patients with AIT and healthy subjects (control group).

We aimed to determine the prevalence and significance of aCL antibodies in children with type 1 diabetes and, for the first time, with AIT.

PATIENTS AND METHODS The study group included patients who were followed up in the Department of Pediatric Endocrinology of the Dokuz Eylul University (Izmir, Turkey) and Gazi University (Ankara, Turkey). There were 74 subjects with type 1 diabetes (mean age 12.91 ± 4.2 years, 40 girls), 64 subjects with AIT (mean age 14.1 ± 3.71 years, 60 girls), and 35 healthy subjects (mean age 12.78 ± 3.32 years, 26 girls). The mean duration (minimum–maximum) of diabetes was 3.62 ± 2.76 (0–13) and AIT was 2.51 ± 2.19 (0.08–8) years. The mean HbA_{1c} value and mean insulin requirement were determined as 8.39 ± 1.84% (5.4–17.4) and 1.3 ± 0.4 IU/kg/day, respectively. The patients' medical records were assessed (adverse reactions to drugs, history of immunosuppressive treatment or chronic disease) and their relatives examined (venous and/or arterial thrombosis/thrombotic microangiopathy, thrombocytopenia, recurrent fetal losses, infections, malignancies, and other autoimmune diseases) to identify any additional medical problems. The subjects underwent thorough physical examination prior to the study. The diagnosis of type 1 diabetes was made based on the World Health Organization criteria and AIT was diagnosed on the basis of a history of typical symptoms or signs of hypothyroidism, including high serum thyrotropin levels and the presence of either antithyroglobulin (anti-TgAb) or antithyroid peroxidase (anti-tPOAb) antibodies (or both) in serum.^{7,13,14} The control group comprised children without any infectious and autoimmune diseases.

The serum levels of anti-TgAb, anti-tPOAb, and aCL antibodies were measured in all patients with type 1 diabetes and AIT regardless of disease duration. The cut-off for a negative result has been defined as <10 IU/ml for both anti-tPOAb and anti-TgAb.

aCL IgG and aCL IgM antibodies were measured with enzyme-linked immunosorbent assay (ELISA) (ZEUS Lübeck, Germany). The results were interpreted using the following Sapporo criteria^{15,16}: low positive, aCL IgG and IgM levels of 20 GPL/ml and 20 MPL/ml, respectively; medium/high positive, aCL IgG and IgM levels above 40 GPL/ml–MPL/ml and 80 GPL/ml–MPL/ml (>99th percentile), respectively.

The age at the time of diagnosis, the duration of diabetes, the doses of insulin according to weight, and the HbA_{1c} levels of patients with diabetes were determined. Cobra Integra 400 plus device (Roche Diagnostics, Shweiz) was

used for measuring the HbA_{1c} levels of diabetic children. Normal HbA_{1c} levels were defined as 4.5%–5.7%.

After providing the legal guardians and families of children with relevant information, a written informed consent was obtained for the study and aCL antibody determination. The study was approved by the Internal Review Board of the Ethics Committee.

The data were analyzed with the SPSS 11.5 package. The Pearson correlation analysis was used to determine whether there was a relation between the duration of disease and aCL antibody positivity. The one-way variance analysis (ANOVA) and χ^2 tests were used to compare the laboratory and clinical parameters between the groups. When ANOVA analysis resulted in a significant difference ($P < 0.05$), the Bonferroni post-hoc test was used to identify a group that accounts for statistical significance ($P < 0.0167$ was considered statistically significant [$P < 0.05/3 = 0.0167$]). All data were expressed as mean ± standard deviation.

RESULTS In the AIT group, the prevalence of anti-TgAb and anti-tPOAb positivity was significantly higher than in subjects with type 1 diabetes or healthy subjects ($P > 0.05$) (TABLE). The positivity rate of aCL antibodies was higher in patients with AIT compared with diabetic or healthy subjects when low positive cut-off level was used (21.9%, 5.4%, 5.7% for aCL IgG and 21.9%, 6.8%, 5.7% for aCL IgM, respectively, $P < 0.05$) (TABLE). However, when we used a medium/high cut-off level, no significant difference was found between the groups (12.5%, 2.7%, 2.8% for aCL IgG and 12.5%, 5.4%, 2.8% for aCL IgM, respectively, $P > 0.05$) (TABLE).

The aCL antibody titers were statistically higher in AIT compared with diabetic or healthy subjects (14.2 ± 28.9, 7.2 ± 16.6, 1.9 ± 6.7 for aCL IgG [GPL/ml] and 13.3 ± 35.3, 3.1 ± 11.6, 1.9 ± 9.3 for aCL IgM [MPL/ml], respectively [TABLE]). No correlation was found between aCL IgG and IgM antibody positivity and the duration of disease ($r = 0.153$, $r = 0.02$ for type 1 diabetes and $r = 0.001$, $r = 0.009$ for AIT, respectively). Three diabetic patients developed microvascular complications (microalbuminuria), but none of them showed aCL antibody positivity.

DISCUSSION aCL antibodies are typically known as the serological markers of APS, an autoimmune disease characterized mainly by venous and/or arterial thrombosis, thrombocytopenia, and complicated pregnancies with recurrent miscarriage.^{17,18} It has been reported that aCL antibody positivity may reach up to 50% in patients with systemic lupus erythematosus and 53% in patients with juvenile rheumatoid arthritis.^{19,20} As for aCL antibodies in type 1 diabetes, only a few studies on small patient populations have been conducted so far.^{1,5,6}

It has been observed that aCL antibody levels were higher in patients with recent-onset

TABLE Clinical and laboratory characteristics of the patient groups

	Type 1 diabetes (n = 74)	AIT (n = 64)	Controls (n = 35)	P
age (year) ^a	12.9 ± 4.2	14.1 ± 3.7	12.8 ± 3.3	0.134
aCL IgG level (GPL/l) ^a	7.2 ± 16.6 ^c	14.2 ± 28.9 ^d	1.9 ± 6.7 ^{c,d}	0.001
aCL IgM level (MPL/l) ^a	3.1 ± 11.6 ^c	13.3 ± 35.3 ^d	1.9 ± 9.3 ^{c,d}	0.013
aCL IgG low positivity (%) ^b	5.4 (4/74)	21.9 (14/64) ^e	5.7 (2/35)	0.005
aCL IgM low positivity (%) ^b	6.8 (5/74)	21.9 (14/64) ^e	5.7 (2/35)	0.011
aCL IgG medium/high positivity (%) ^b	2.7 (2/74)	12.5 (8/64)	2.8 (1/35)	0.101
aCL IgM medium/high positivity (%) ^b	5.4 (4/74)	12.5 (8/64)	2.8 (1/35)	0.563
anti-TgAb (%) ^b	20.3 (15/74) ^c	89.1 (57/64) ^d	5.7 (2/35) ^{c,d}	0.001
anti-tPOAb (%) ^b	24.3 (18/74) ^c	93.7 (59/64) ^d	8.6 (3/35) ^{c,d}	0.001
anti-TgAb and anti-tPOAb (%) ^b	16.2 (12/74) ^c	85.9 (55/65) ^d	2.9 (1/35) ^{c,d}	0.001

a one-way ANOVA

b χ^2 test

c statistically significant difference ($P < 0.016$) between the group with type 1 diabetes and healthy subjects

d statistically significant difference ($P < 0.016$) between the group with AIT and healthy subjects

e statistically significant difference originates from the patients with AIT

Abbreviations: aCL – anticardiolipin antibody, AIT – autoimmune thyroiditis, anti-TgAb – antithyroglobulin antibodies, anti-tPOAb – antithyroid peroxidase antibodies, Ig – immunoglobulin

diabetes.⁶ Galtier-Dereure et al.¹ assessed the prevalence of aCL antibodies in diabetes with and without complications and found aCL antibodies in 9.4% and 9.5% of type 1 and type 2 diabetic patients, respectively. They also found a higher prevalence of these antibodies in diabetic patients with macroangiopathy (32.4%) or overt nephropathy (32.1%) and suggested a potential role of aCL antibodies in the progression of diabetic vascular complications. However, a high rate of aCL antibody positivity in patients with nephropathy was not related to primary renal pathology. Mohamed et al.⁶ demonstrated that the mean levels of serum aCL antibodies were significantly higher in subjects with type 1 diabetes, even in very young diabetics without complications, than in healthy subjects. In addition, they found a negative correlation between the duration of diabetes and aCL antibody levels. Lorino et al.⁵ detected aCL IgG antibody positivity in 24% (7/29) of diabetic patients, while aCL IgM antibody positivity was not detected at all. Ahmed et al.⁹ reported an increased prevalence of aCL IgM antibodies in diabetic adults with vascular complications, but not in patients without vascular complications and healthy subjects. In the same study, the prevalence of aCL IgG positivity was determined as 21% (8/38) in type 2 diabetes and 4.9% (5/102) in healthy subjects. The present study, in which no statistical significance was found between type 1 diabetes and healthy subjects regarding aCL antibody positivity, suggested that screening for aCL antibodies in diabetics has no clinical importance. Likewise, the positivity of certain antibodies in diabetic patients does not always indicate organ dysfunction. For example, the majority of diabetic patients with antithyroidal antibody positivity do not experience hypothyroidism. Therefore, aCL positivity does not determine the development of microvascular complications.

These data raised the question whether aCL antibody positivity could be a predictor of late-onset micro- and macrovascular complications. However, long-term follow-up studies that evaluate patients with aCL antibody positivity are required to demonstrate such a relationship.

It has also been shown previously that aCL antibody positivity was not associated with good metabolic control in diabetic patients.⁵ However, Mohammed et al.⁶ reported a positive correlation between diabetes duration and aCL antibody titer. Such relationship was not confirmed in the current study.

So far, researchers have provided inconsistent data on the prevalence of aCL antibody positivity in AIT.^{7,8,12} The cause of this prevalence still remains unknown.^{7,11} However, it has been reported that the positivity might be an unspecific indicator of autoimmune dysregulation.^{7,8} Osundeko et al.⁷ studied IgA, IgG, and IgM aCL antibodies in 19 patients with isolated AIT aged between 26 and 79 years and found the positivity rate of 21% (IgG in 2 and IgM in 2 cases). On the other hand, Diez et al.¹² determined aCL antibody positivity in 10.1% (7/69) of AIT patients (Graves' disease and Hashimoto's thyroiditis). However, this rate was similar to that detected in non-AIT subjects (11.2%) and healthy subjects (4.1%). Paggi et al.⁸ reported that patients with uncontrolled and severe Graves' disease had high levels of aCL antibodies, which decreased after methimazole therapy.⁸ In our study, the presence of both aCL IgG and IgM antibodies was detected at the rate of 21.9% in the AIT group when low positive aCL cut-off level was used, which was higher than in other studies. Nevertheless, none of our patients had any symptoms or signs of APS and no correlation was found between the presence of antibodies and duration of AIT. A number of studies have suggested that the clinical

features of APS are associated with high levels of IgG aCL and only about 30% of individuals with antiphospholipid antibodies develop the clinical syndrome.^{7,21} Accordingly, when we used medium/high cut-off levels, the frequency of antibody positivity was similar between the groups. All of these findings led us to consider that elevated aCL antibody levels in AIT are a nonspecific indicator of autoimmune dysregulation.

Our study has several limitations. First, the presence of aCL positivity in 2 or more samples obtained at least 12-week intervals is required to diagnose APS,¹⁵ while we were able to perform only 1 aCL measurement and unable to study lupus anticoagulant or antibodies against β -2-glycoprotein 1. Second, aCL antibody may also be positive in some infectious diseases, such as syphilis, human immunodeficiency virus (HIV), and hepatitis C virus (HCV) infections.¹⁸ However, we did not investigate positivity for syphilis, HCV or HIV infection due to a lack of indications in medical history and clinical findings.

In conclusion, our study showed a high prevalence of aCL antibody positivity in patients with AIT at a low-positive aCL cut-off level. However, at a medium/high aCL cut-off level, the rate of aCL antibody positivity between the groups was similar. In addition, the rate of positivity of aCL antibodies did not correlate with clinical findings in patients with AIT and type 1 diabetes. For all of these reasons, we believe that a routine investigation of aCL antibodies in childhood may not have a clinical significance for diabetic and AIT patients without vascular or clinical symptoms. However, studies with a long-term follow-up of these patients are needed to determine a possible association between aCL antibodies and vascular complications that may develop in the later stages of the disease.

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Występowanie przeciwciał antykardiolipinowych w cukrzycy typu 1 oraz autoimmunologicznym zapaleniu tarczycy

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SŁOWA KLUCZOWE

autoimmunologiczne zapalenie tarczycy, cukrzyca, przeciwciała antykardiolipinowe

STRESZCZENIE

WPROWADZENIE Wysokie stężenie przeciwciał antykardiolipinowych może być wskaźnikiem powikłań naczyniowych w cukrzycy typu 1 oraz autoimmunologicznym zapaleniu tarczycy (*autoimmune thyroiditis* – AIT). Znaczenie kliniczne tych przeciwciał u chorych na cukrzycę typu 1 lub AIT pozostaje niejasne.

CELE Celem badania była analiza występowania przeciwciał antykardiolipinowych oraz ich znaczenia u chorych na cukrzycę typu 1 lub AIT.

PACJENCI I METODY W badaniu wzięło udział 74 pacjentów z cukrzycą typu 1 (średni wiek $12,9 \pm 4,2$ roku), 64 pacjentów z AIT (średni wiek $14,1 \pm 3,7$ roku), oraz 35 zdrowych osób z grupy kontrolnej (średni wiek $12,8 \pm 3,3$ roku). Stężenia przeciwciał antykardiolipinowych w klasach immunoglobulin (Ig) G oraz IgM oznaczono za pomocą testów immunoenzymatycznych. Poziomy przeciwciał antykardiolipinowych u chorych wykazujących ich obecność sklasyfikowano w 2 kategoriach jako niskie oraz umiarkowane/wysokie.

WYNIKI Niski poziom przeciwciał aCL występował częściej u chorych z AIT niż u chorych na cukrzycę i osób zdrowych ($P < 0.05$), podczas gdy nie zaobserwowano statystycznie istotnej różnicy w częstości występowania umiarkowanego/wysokiego poziomu aCL pomiędzy chorymi z AIT oraz cukrzycą a grupą osób zdrowych.

WNIOSKI Badanie wykazało większą częstość występowania niskich poziomów przeciwciał antykardiolipinowych u chorych z AIT. Jednocześnie nie obserwowano istotnej różnicy w częstości występowania umiarkowanych/wysokich poziomów przeciwciał antykardiolipinowych pomiędzy poszczególnymi grupami. Uważamy, że rutynowe oznaczanie stężenia przeciwciał antykardiolipinowych nie ma znaczenia klinicznego u dzieci z cukrzycą typu 1 oraz AIT.

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