Anticardiolipin Antibodies in Children With Insulin-Dependent Diabetes Mellitus

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Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease that selectively destroys insulin-secreting pancreatic β cells. Anticardiolipin antibody is an autoantibody directed against cell membranes. An association of this antibody with diabetes mellitus has not been widely reported. The current investigation was performed to determine the prevalence of IgG and IgM anticardiolipin antibodies in 2 groups of children with type 1 IDDM and to find a relation, if any, with the control and duration of the disease. The study included 30 children with type 1 IDDM and 20 healthy control children. The children were subjected to history taking, clinical examination, and laboratory estimation of anticardiolipin IgG and IgM antibodies and glycosylated hemoglobin (HbA_{1c}). Analysis of the results showed that the mean levels of serum anticardiolipin IgG and IgM antibodies were significantly higher among the diabetic children than the healthy controls. Mean values of serum anticardiolipin IgG and IgM antibody levels were significantly higher in children with recent-onset diabetes (29.90 ± 12.60 GPL/mL and 12.825 ± 3.762 MPL/mL) than in those of long duration (>1 year: 10.84 ± 5.796 GPL/mL and 4.142 ± 2.910 MPL/mL, respectively). A negative correlation between the duration of diabetes and the level of IgG and IgM anticardiolipin antibodies and a positive correlation between the level of IgG and the level of IgM antibodies were observed. However, there was a nonsignificant correlation between anticardiolipin IgG and IgM levels and HbA_{1c} levels, insulin dose, and fasting blood sugar. Therefore, anticardiolipin antibodies should be added to the list of autoantibodies detected in IDDM especially in the early stage of the disease.

TYPE 1 (INSULIN-DEPENDENT) diabetes mellitus (IDDM) is attributed to autoimmunity by reason of disease associations, autoantibodies to pancreatic islet cell antigens, similarities with animal models of IDDM, and human leukocyte antigen (HLA) linkages.[1]

Anticardiolipin antibodies were described in the early 1980s.[2] They are directed against negatively charged phospholipids and have been found in systemic lupus erythematosus (SLE),[3] as well as in a variety of other autoimmune diseases and in "primary" antiphospholipid antibody syndrome.[4] Recently, Anzai et al[5] found anticardiolipin antibodies in AKR/J mice with streptozocin-induced diabetes; however, a relationship between anticardiolipin antibodies and diabetes mellitus has not been widely reported.

The aim of the current investigation was to study the prevalence of IgG and IgM anticardiolipin antibodies in children with IDDM (1 group with recent onset and another with a duration >1 year) and to find a relation, if any, with the control of the disease.

MATERIALS AND METHODS

Subjects

This case-control study was conducted on 50 children (30 with type 1 IDDM with a duration of diabetes ranging from 6 months to 7 years) and 20 normal control children. Subjects ranged in age from 4 to 12 years. Diabetic children were receiving care at the Alexandria University Children's Hospital, Alexandria, Egypt. They were18 boys and 12 girls with a mean age of 8.2 ± 4.2 years. The Researchers Committee of the Alexandria Faculty of Medicine approved the study and informed consent to participate in the study was obtained from the caregivers of each child.

Diabetic children were receiving insulin therapy either as Mixtard or NPH (Novo Nordisk, Bagsvaerd, Denmark) and regular insulin in 2 injections daily. The state of glycemic control was evaluated according to subjects' glycosylated hemoglobin (HbA_{1c}) level at the time of the study.

Another 20 apparently healthy children (8 boys and 12 girls) with a mean age of 6.4 ± 3.3 years were included as a control group. The control children had no history of diabetes, or endocrine, collagenic, immune, inflammatory, viral, or malignant diseases that might interfere with the results of tests to determine serum anticardiolipin antibody levels.

Neither cases nor control children were taking any drugs, such as phenothiazines, chlorpromazine, procainamide, hydralazine, or dilantin, that might affect serum levels of anticardiolipin IgG and IgM antibodies (except insulin for the diabetic cases). Blood urea and serum creatinine were in the normal range for both diabetic children and controls.

Methods

Before receiving any treatment, the study population was subjected to clinical assessment and laboratory investigations.

Diabetic children underwent a detailed history taking and full clinical examination that included assessment of growth, presence of complications, age of onset, duration of disease, number of previous admissions with diabetic ketoacidosis, number of hypoglycemic attacks, and the type and dose of insulin therapy received daily. The control children underwent a detailed history and examination.

Laboratory investigations included determination of HbA_{1c} for diabetic cases only.[6] HbA_{1c} was measured using the Helena GLYCO-Tek Affinity Column method, supplied by the manufacturer (Helena Laboratories, catalog no. 5351, Beaumont, TX). The reference range for HbA_{1c} was 4.3% to 7.7%.

The prevalence of IgG and IgM anticardiolipin antibodies was determined in both cases and controls using the enzyme-linked immunosorbent assay (ELISA) technique.[7] The immunoenzymatic kit used for this assay was supplied by BioChem ImmunoSystems (Reno, Italy). One GPL unit is equivalent to 1 μg/mL of an affinity-purified standard anticardiolipin IgG antibody sample and 1 MPL unit is equivalent to 1

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μg/mL of an affinity-purified standard anticardiolipin IgM antibody sample. Values of IgG and IgM anticardiolipin antibodies up to 10 GPL/mL and 10 MPL/mL are considered normal. Values up to 30 GPL/mL (30 MPL/mL) are considered weak positives. Medium positive values go up to 100 GPL/mL (100 MPL/mL). Values above 100 GPL/mL (100 MPL/mL) are considered highly positive.[7-9]

Other laboratory investigations were renal function tests (serum urea and creatinine) for the cases,[9] and fasting blood glucose levels for both cases and controls.[9]

Data were analyzed using the SPSS 6.1 statistical software package (SPSS Inc, Chicago, IL) and are expressed as the mean ± SD. Student's *t* test was also used to compare means of biochemical parameters.

RESULTS AND DISCUSSION

The demographic features (age and gender) that could affect the results were comparable in diabetic children and controls ($t = 1.63$ and $\chi^2 = 1.2$, $P \geq .05$) None of diabetic children had evidence of complications of IDDM such as microalbuminuria or vascular indications, except for 4 cases who had a history of repeated attacks of ketoacidosis and/or hypoglycemia and frequent admission to the hospital for control of the disease.

Anticardiolipin antibodies were identified and measured in the serum of healthy control children and ranged from 2.2 to 10.9 GPL (mean, 4.24 ± 2.29 GPL) for IgG anticardiolipin antibodies and 1.7 to 9.0 MPL (mean, 3.14 ± 1.78 MPL) for IgM anticardiolipin antibodies (Table 1), without a significant difference between boys and girls. Thus, gender had no influence on serum anticardiolipin antibodies in healthy children. Tietjen et al[10] reported a mean value of 8.3 ± 14.8 GPL for IgG anticardiolipin antibodies in control adult subjects, which is higher than the mean level detected in healthy children of the present study, perhaps due to the difference in age.

The results of the present study showed that 53.3% and 16.6% of type I diabetic children were positive for IgG and IgM anticardiolipin antibodies, respectively, which was higher than the percentages of children having positive tests for IgG and IgM anticardiolipin antibodies among the control group (5% and 0%, respectively; $\chi^2 = 10.4$ and 2.08, respectively)

The results of the present study also showed that children with type 1 IDDM had IgG anticardiolipin antibodies, with a range between 3.9 to 45.7 GPL (mean ± SD, 13.38 ± 9.46 GPL); the range of IgM anticardiolipin antibodies was 1.1 to 18.2 MPL (5.39 ± 4.25 MPL). These results were found to be significantly higher than those of the control group ($t = 4.43$ and 2.23 , $P \leq .0001$ and $<.05$, respectively). Figures 1 and 2 show dot plots for IgG and IgM anticardiolipin antibodies in both diabetic cases and controls.

Table 1. IgG and IgM Anticardiolipin Levels of All Diabetic Children and Controls.

Anticardiolipin Levels	Cases (n = 30)	Controls (n = 20)	Significance
IgG (GPL/mL)			
Range	3.9-45.7	2.2-10.9	
Mean	13.3833	4.2400	$t = 4.433$
SD	9.4638	2.292	$P = .00005$*
IgM (MPL/mL)			
Range	1.1-18.2	1.7-9.0	
Mean	5.3900	3.1400	$t = 2.2336$
SD	4.255	1.787	$P = .03$†

*Highly significant.

†Significant, $P < .05$.

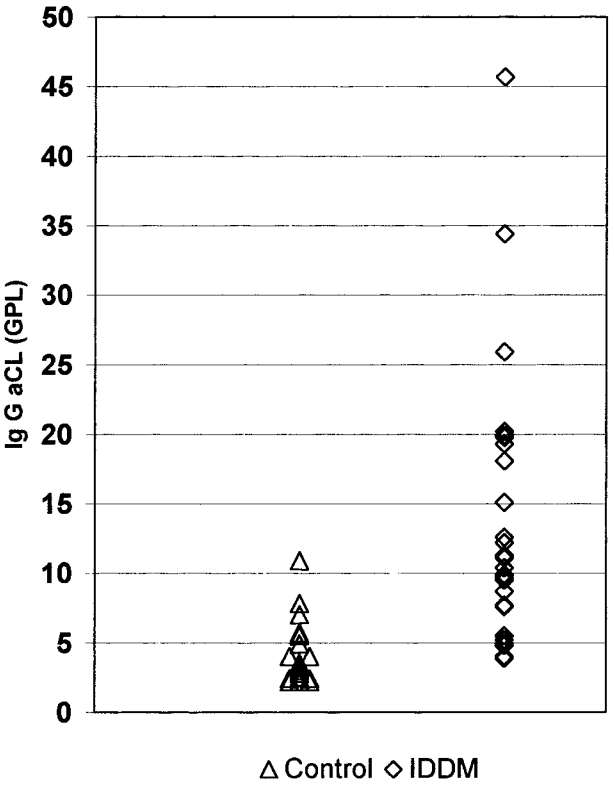

Fig 1. IgG anticardiolipin antibodies (aCL) in diabetic patients and controls.

Anticardiolipin antibodies, specifically those of the IgG isotype, are an independent risk factor for ischemic stroke, and data from several studies suggest they may play their most prominent role in cerebral ischemia in young adults. Tietjen et al[10] reported a mean level of IgG anticardiolipin antibodies for transient focal neurologic events of 7.8 ± 14 GPL and a range from 0 to 16.75 GPL. Anticardiolipin antibodies have been identified in approximately 10% of unselected patients with first ischemic stroke. The mechanism by which anticardiolipin antibodies and other acquired circulating antibodies (eg, the lupus anticoagulant) induce ischemia has not been established with certainty.

Roubey[11] reported the prevalence of anticardiolipin antibodies in the general population to be from 0% to 14%, with most studies citing a prevalence of less than 5%,[7] similar to our results for the control group. Roubey also identified anticardiolipin antibodies in 18% to 61% of patients with SLE and in 8% to 33% of patients with rheumatoid arthritis.[7] Another study also showed that 50% of children with SLE had antiphospholipid antibodies[12] and 53% of children with juvenile rheumatoid arthritis were positive for IgG and IgM anticardiolipin antibodies.[13]

Caporali et al[4] studied the prevalence of anticardiolipin an-

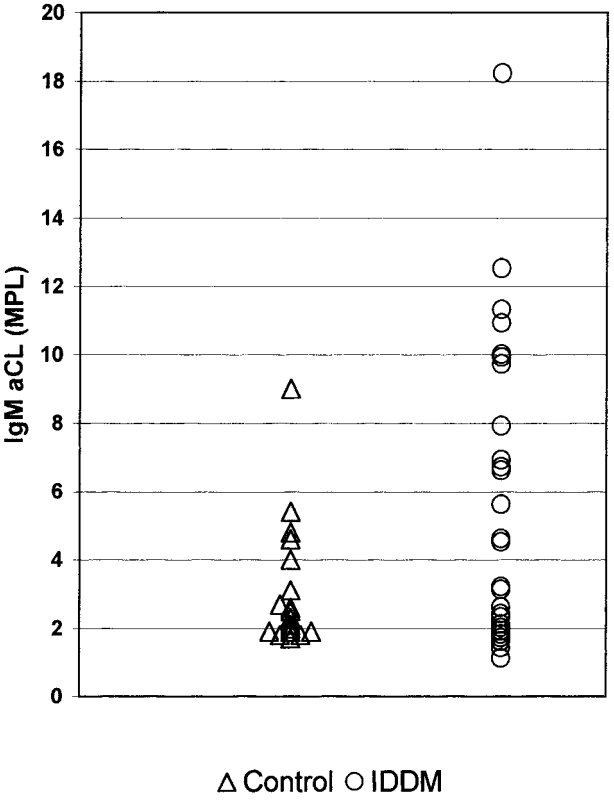

Fig 2. IgM aCL in diabetic patients and controls.

tibodies in 70 children with juvenile chronic arthritis and 25 adult patients with rheumatoid arthritis. Fifty-three percent of patients were positive for IgG or IgM antibodies or both. IgG anticardiolipin antibodies were negative in all control subjects, while IgM antibodies were detected in 5% of children and 4% of adult controls. It can be noted that Caporali et al[4] showed a higher percentage of positive tests for IgM anticardiolipin antibodies among the controls than in the present study (5% *v* 0%). Caporali et al reported no correlations in patients with juvenile chronic arthritis between the presence of titers of anticardiolipin antibodies and various clinical and laboratory variables. No patient with anticardiolipin antibodies showed any feature of the anticardiolipin syndrome. They also noted that all patients with a high titer of IgG anticardiolipin antibodies had an increased erythrocyte sedimentation rate (ESR). This finding might suggest that these antibodies are present in high titer only in patients with active disease, although other studies reported no correlation between anticardiolipin antibodies and ESR or disease activity defined on clinical grounds.[7] It can be noted that the percentage of diabetic children with a positive test for IgG anticardiolipin antibodies was comparable to that reported for many autoimmune diseases.

In the present study, 16.6% of diabetic cases were weakly positive for IgM anticardiolipin antibodies (>10 MPL/mL). None of the cases had values greater than than 30 MPL/ml, indicating that none were moderately or highly positive. Our study also showed that 46.6% of cases were weakly positive for IgG anticardiolipin antibodies, while only 1 of 30 cases was moderately positive (>30 GPL) and 1 of 30 had a level greater than 40 GPL, although none showed evidence of vascular complications or any manifestation of the antiphospholipid syndrome. Complications due to antiphospholipid antibodies may depend on both the presence of the antibody and another, as yet unidentified, inciting factor or conditions. The younger age of the population of the present study may be another factor. Lorini et al[14] studied 30 IDDM patients aged 3.9 to 26.8 years with a duration of diabetes that ranged from 1 month to 19 years. They reported that IgG anticardiolipin antibodies were found in 7 patients (24%) and were more frequent in IDDM patients than in controls ($P < .005$). IgM anticardiolipin antibodies were absent in all diabetic cases. This may be due to the higher cut-off levels used in this study. However, they reported a 5% percentage of positive tests for IgM anticardiolipin antibodies among the control group, which is similar to that reported by Caporali et al[4] and higher than the results of the present study (0%).

Levine et al[8] reported that subsequent thrombo-occlusive events and death after focal cerebral ischemia associated with IgG anticardiolipin antibodies may occur sooner and more frequently with a GPL greater than 40. Patients with a GPL greater than 40 also appeared to have more features of antiphospholipid syndrome: younger age at onset and more recurrent strokes at the time of diagnosis.

In addition, Ginsburg et al,[15] Watanabe et al,[16] and Finazzi et al[17] found that a titer of GPL greater than 33 identified a group at increased risk of subsequent deep vein thrombosis and pulmonary embolism. Others have found that higher GPL titers may confer an increased risk of fetal loss.[16,17]

In the present study, the duration of diabetes ranged from 6 months to 7 years, with a mean of 2.82 ± 1.37 years. Diabetic children were divided into 2 subgroups: group I (short duration) comprised children who had a recent onset of diabetes (<1 year), while group II (long duration) comprised children who had diabetes for more than 1 year.

The results of the present study showed that on studying the level of IgG anticardiolipin antibody in children examined during the first year after diagnosis of IDDM, significantly higher serum anticardiolipin antibody levels were detected than among those with a duration ≥ 1 year (Table 2). In group I cases positive for IgG anticardiolipin antibody, the mean level was 29.9 ± 12.6 GPL, while in cases positive for IgM anticardiolipin antibody, the mean was 12.8 ± 3.7 MPL. On the other

Table 2. Serum Anticardiolipin IgG and IgM Levels in Diabetic Subgroups (short duration and long duration) and Control Group

Anticardiolipin Levels	Short Duration, <1 yr (n = 4)	Long Duration, >1 yr (n = 26)	Significance
IgG (GPL/mL)			
Range	19.3-45.7	3.9-25.9	
Mean	29.900	10.842	$t = 5.1754$
SD	±12.601	5.796	$P = .000016$*
IgM (MPL/mL)			
Range	9.7–18.2	1.6–11.3	
Mean	12.825	4.1423	$t = 5.366$
SD	3.762	2.910	$P = .00001$*

*Highly significant.

hand, in group II, the mean IgG anticardiolipin antibody level was 10.8 ± 5.79 and the mean IgM anticardiolipin antibody level was 4.1 ± 2.9 MPL (t = 5.175 and 5.366, $P \leq .0001$)

In the present study, 100% of children with type I IDDM and a disease duration less than 1 year were positive for IgG anticardiolipin antibodies and 46.1% of children with a duration of more than 1 year were positive compared to only 5% of controls.

As regards IgM anticardiolipin antibodies, 75% of diabetic children with a disease duration less than 1 year were positive, while only 7.6% of those with a disease duration more than 1 year had positive tests compared to 0% of the controls.

In harmony with our results, Lorini et al[14] reported a higher frequency of anticardiolipin IgG antibodies among recently diagnosed IDDM children and adolescents with a disease duration up to 6 months (54.5%) compared to controls (0%) and group II patients with a duration of more than 6 months (5.5%). On the other hand, they reported low IgM levels in recently diagnosed group I patients with a duration of IDDM less than 6 months (0%) compared to controls (5%). The results of IgM anticardiolipin antibodies of the present study showed a higher percentage of recent-onset diabetics with positive tests (75%) and lower percentage among controls (0%). These findings point to the possible role played by IgM anticardiolipin antibodies in the autoimmune process of early diabetes. The difference in results of Lorini et al[14] and the present study may be caused by the somewhat older ages of diabetic cases and controls in Lorini's study (range, 3.9 to 26.8 years v 4 to 12 years in the present study), in addition to the longer duration of diabetes (1 month to 19 years v 6 months to 7 years in the present study). These findings may indicate the role played by IgM anticardiolipin antibodies in diabetics of younger age and shorter duration. However, Lorini et al[14] did not find positive IgM anticardiolipin antibody tests even in recent-onset IDDM. Whether this difference was due to their older ages than in the present study cannot be answered, because Lorini et al did not provide the ages of their subjects. In addition, the small number of recent-onset diabetics in the present study (n = 4) indicates that results should be confirmed by a larger study. Another question that should be answered by further studies is whether these children with positive tests for IgM anticardiolipin antibodies will be more vulnerable to vascular complications in the future. Ahmed et al[18] reported an increased prevalence of IgM anticardiolipin antibodies in diabetic adults with vascular complications, but not in patients without vascular complications and controls. On the other hand, D'Annunzio et al[19] reported that the rate of IgM anticardiolipin antibodies was similar in first-degree relatives of diabetic patients and controls. They found positive tests in 2 of 42 siblings and 2 of 52 of controls, all in the low-positive group. These findings may indicate that IgM anticardiolipin antibodies play a role in some cases of IDDM either of recent onset or with longer duration. This may depend on age, duration of diabetes, vascular complications, genetic or racial differences, or other unidentified factors.

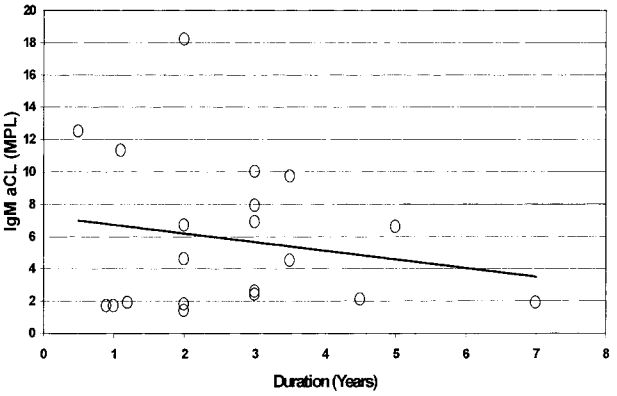

Fig 4. Correlation between duration of IDDM and IgM aCL.

Furthermore, the results of the present study showed a negative correlation between the duration of IDDM and the levels of IgG and IgM anticardiolipin antibodies (r =-0.58 and -0.46, respectively) and a positive correlation between both antibodies (r = 0.685) (Figs 3 through 5) D'Annunzio et al[19] reported that in animal models in which diabetes was induced by streptozocin, anticardiolipin antibodies appeared when hyperglycemia began to occur, increased when diabetes developed, and then fell. The authors suggested that anticardiolipin antibodies might be produced in association with the development of insulinitis, but not induced by hyperglycemia or streptozocin.[14]

Furthermore, D'Annunzio et al[19] observed higher levels of

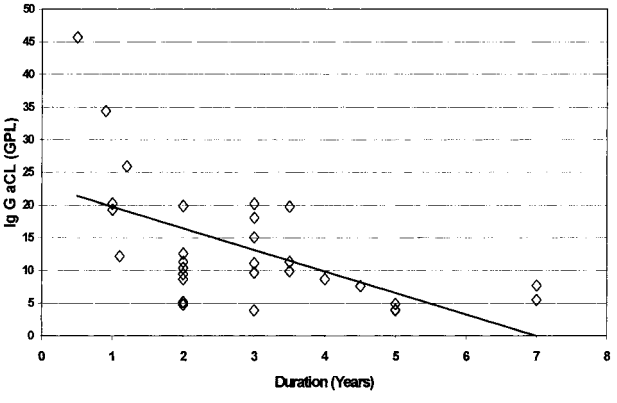

Fig 3. Correlation between duration of IDDM and IgG aCL.

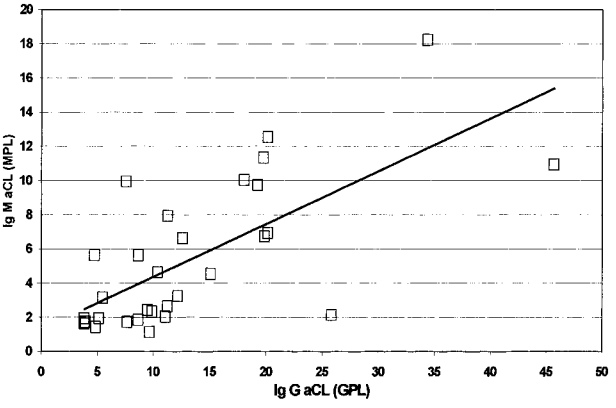

Fig 5. Correlation between IgG aCL and IgM aCL in IDDM patients.

IgG anticardiolipin antibodies in first-degree relatives of IDDM patients than in controls; they suggested that the presence of anticardiolipin antibodies in siblings of diabetic patients may represent a nonspecific marker of autoimmunity, with increased production of several antibodies against different antigens, as described in subjects at risk of IDDM. They hypothesized that anticardiolipin antibodies may constitute a feature of genetic predisposition to that disease.

An association between high frequency of antiphospholipid positivity and pregnancy complications has been reported, which strongly supports a major role for an autoimmune pathogenetic mechanism in the occurrence of feto-maternal morbidity in IDDM pregnant women.[20]

In the present study, as regards glycemic control of IDDM children, the level of HbA_{1c} ranged from 4.2% to 11.4% (mean ± SD, 8.01% ± 1.6%).The level of HbA_{1c} was between 6% and 9% in 77% of IDDM patients, denoting good control, while 23% had levels between 9% and 12%, denoting fair control. None of the IDDM patients had levels greater than 12%, which meant that none were poorly controlled.

No correlation was found between HbA_{1c} level or insulin dose or fasting blood sugar and IgG and IgM anticardiolipin antibody levels. No significant difference was found in anticardiolipin antibody levels between diabetic children classified according to metabolic control as either good or fair. Similar findings were reported by Lorini et al,[14] D′Annunzio et al,[19] and Clark et al.[20]

An association of anticardiolipin antibodies with diabetes mellitus may reflect the autoimmune nature of both conditions or the alteration of the vascular endothelium, which may stimulate anticardiolipin antibody formation. The presence of anticardiolipin antibodies in diabetic patients, particularly newly diagnosed subjects, may reflect an abnormal immunologic response in the early stage of diabetes with increased production of autoantibodies against different organs. It confirms that the presence of anticardiolipin antibodies is a secondary autoimmune phenomenon, to be added to the multitude of antibodies already described at the onset of type I diabetes.

CONCLUSIONS

It can be concluded that there are high levels of IgG and IgM anticardiolipin antibodies in children with IDDM and that the serum IgG and IgM anticardiolipin antibody level has a negative correlation with the duration of diabetes, being higher in subjects with disease of recent onset. Therefore, anticardiolipin antibodies may reflect an abnormal immunologic response in IDDM, especially in the early stage of the disease, and should be added to the list of antibodies detected in IDDM.

Recommendations

Detection and measurement of IgG and IgM anticardiolipin antibody level is noninvasive and fast. These advantages may facilitate early detection among subjects at risk of developing IDDM, particularly relatives of patients, who are at higher risk than the general population.

Further studies should be done to clarify the regulatory mechanism and the precise function of anticardiolipin antibodies in IDDM and its relation to complications. In addition, studies should be performed to clarify the ability to antagonize anticardiolipin antibodies, which may provide a novel therapeutic approach to preventing IDDM.

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