High prevalence of immunoglobulin A deficiency in patients with type 1 diabetes mellitus detected by ELISA

Alta prevalência de deficiência de imunoglobulina A detectada por ELISA em pacientes com diabetes mellitus

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ABSTRACT

Objective: To measure serum levels of immunoglobulin A by immunoenzymatic assay (ELISA) in type 1 diabetes mellitus (DM-1) patients and to verify the prevalence of immunoglobulin A deficiency (IgAD) in diabetic patients. Methods: The serum immunoglobulin A level was determined in 149 DM-1 patients by three methods. IgAD was defined as serum immunoglobulin A level lower than 5 mg/dl. If serum immunoglobulin A level was undetectable by turbidimetry, radial immunodiffusion was performed in low plate concentration. For patients with undetectable serum immunoglobulin A level by the two previous methods, quantification was performed by ELISA. In patients with IgAD, the levels of immunoglobulins G and M were measured by turbidimetry to exclude other humoral immunodeficiencies. Results: Out of 149 DM-1 patients evaluated, 141 (94.6%) had normal serum immunoglobulin A levels by turbidimetry. Eight patients (5.3%) had undetectable serum immunoglobulin A levels by turbidimetry and radial immunodiffusion. In these eight patients, the determination of serum immunoglobulin A was performed by ELISA, a more sensitive method. Very low levels of serum immunoglobulin A were detected in these diabetic patients. In all diabetic patients, immunoglobulins G and M were normal for age by turbidimetry. All 150 patients of the Control Group had normal serum immunoglobulin A levels by ELISA. Conclusions: There was a significantly higher prevalence of immunoglobulin A deficiency among DM-1 patients (5.3%). Measurement of serum immunoglobulin A is necessary in all DM-1 particularly before some immunoglobulin A antibody screening. Patients with IgAD may have false-negative results for celiac disease screening tests involving immunoglobulin A antiendomysium and antigliadin antibodies.

Keywords: Immunoglobulin A; Diabetes mellitus, type 1; Enzyme-linked immunosorbent assay

RESUMO

Objetivo: Medir as concentrações séricas de imunoglobulina A por ensaio imunoenzimático (ELISA) em pacientes com diabetes mellitus tipo 1 (DM-1) e verificar a prevalência da deficiência de imunoglobulina A (DlGA) em pacientes diabéticos. Métodos: A concentração sérica de imunoglobulina A foi determinada em 149 pacientes portadores de DM-1 por três métodos. A DlGA foi definida como sendo nível sérico inferior a 5 mg/dl. Quando os níveis séricos de imunoglobulina A eram indetectáveis por turbidimetria, realizou-se imunodifusão radial em concentração de placa baixa. Para os pacientes em que os níveis séricos de imunoglobulina A foram indetectáveis pelos dois métodos anteriores, a imunoglobulina sérica foi quantificada por ELISA. Em pacientes com DlGA, os níveis de imunoglobulina G e M foram medidos por turbidimetria para excluir outras deficiências humorais. Resultados: Dos 149 pacientes portadores de DM-1 avaliados, 141 (94,6%) tinham níveis séricos normais de imunoglobulina A por turbidimetria. Oito pacientes (5,3%) apresentavam níveis séricos indetectáveis de imunoglobulina A por turbidimetria e imunodifusão radial. Nesses oito pacientes, a determinação sérica de imunoglobulina A foi realizada por um método mais sensível, ELISA. Níveis séricos muito baixos de imunoglobulina A foram detectados nesses pacientes diabéticos. Em todos os pacientes diabéticos, os níveis séricos de imunoglobulinas G e M medidos por turbidimetria foram normais para a idade. Todos os 150 pacientes do Grupo Controle tiveram níveis séricos normais de imunoglobulina A por ELISA. Conclusões: Houve uma prevalência significativamente alta de DlGA entre os pacientes diabéticos tipo 1 (5,3%). A dosagem de imunoglobulina A em todos os pacientes diabéticos do tipo 1 é necessária, especialmente na fase anterior a alguns testes de triagem baseados em anticorpos de imunoglobulina A. Pacientes com DlGA podem ter resultados falsos-negativos para testes de triagem de doença celiaca envolvendo anticorpos antidiomiossími e antigliadin.

Descritores: Imunoglobulina A; Diabetes mellitus tipo 1; ELISA
INTRODUCTION

Immunoglobulin A deficiency (IgAD) is defined as serum IgA levels lower than 5 mg/dl followed by normal or increased levels of serum immunoglobulin G (IgG) and M (IgM)\(^9\).

IgAD is the most common primary immunodeficiency, with a reported prevalence between 1:223 and 1:1000 in different countries\(^2\)-\(^4\). The prevalence of IgAD in the Brazilian population is 1:965\(^5\).

In patients with IgAD, the initial B-cell differentiation pathways are intact and there is a normal expression of surface IgA. The immunologic disturbance is possibly located in IgA producing plasmocytes\(^6\)-\(^7\). These cells are present in IgAD patients, but in reduced amount, and their phenotype is immature. Mature B cells express surface IgA, IgM and IgD\(^8\).

Immunoglobulin A is important to control the exposure of the immune system to ingested antigens. The immune system associated with the mucosa produces specific antibodies in order to delay the transport of ingested antigens to circulation. The mechanism is thought to occur by means of IgA antibodies that prevent binding of antigens to epithelial cells\(^9\).

The IgA molecule has the capacity to combine with exogenous antigens, avoid their penetration into circulation and trigger an immunologic response\(^10\). If antigens enter circulation, they stimulate the development of antibodies and cause a cross reaction with tissue-specific self-antigens, possibly resulting in other immunologic events, such as autoimmune diseases\(^11\).

The absence of the mechanism to eliminate antigens, normally performed by IgA in the mucosal membranes, allows greater antigenic exposure to the lymphatic system and modifies the control of T-lymphocytes and formation of antibodies, leading to greater prevalence of autoimmune diseases in IgAD patients\(^12\).

There is evidence of these facts, such as type 1 diabetes mellitus (DM-1) and celiac disease (CD) in patients with IgAD\(^13\)-\(^14\). CD has a well-established association with IgAD and is considered the most common noninfectious intestinal disorder in IgAD patients\(^14\).

Although the exact mechanism and familiar pattern of inheritance are still unknown, greater susceptibility to DM-1 has been associated to various genes encoding for immune response, including HLA class I (HLA-A, B, C), class II (HLA-DR, DQ, DP) and class III genes\(^15\).

OBJECTIVE

The objectives of the present study were to measure the serum concentration of IgA in DM-1 patients, to determine the prevalence of IgAD among these patients and to perform an immunoassay to detect serum IgA in concentrations lower than 5 mg/dl.

METHODS

A highly sensitive enzyme-linked immunosorbent assay (ELISA) was developed since conventional laboratory methods only allowed determination of serum IgA levels ranging from 0.84 to 1.3 mg/dl by radial immunodiffusion (RID) and from 40 to 500 mg/dl by turbidimetry. Confirming IgAD is necessary to avoid false-negative serological tests, as it would occur in DM-1 patients with other autoimmune diseases. For example, CD screening is based on IgA antigliadin and IgA antiendomysium antibodies. This study recommends the use of ELISA as a more sensitive method to determine serum IgA levels.

A total of 149 DM-1 children and young adults were evaluated in follow-up at Hospital de Clínicas of the Universidade Federal do Paraná (UFPR). All patients and/or guardians answered a questionnaire to evaluate the presence of IgAD signs and symptoms, history of respiratory and gastrointestinal infection and duration of breast feeding. An informed consent was obtained from all patients and/or guardians.

All patients were examined and a blood sample was collected for further analysis. There were 77 (51.7%) males and 72 (48.3%) females. The age range was 1.7 to 22.5 years. The mean age was 12.6 ± 4.0 and the median age was 12.9 years. The mean age at onset of DM-1 was 7.0 ± 3.5 years (median of 6.8 years) and the mean duration of DM-1 was 5.4 ± 4.2 years (median of 4.6 years).

The Control Group consisted of 150 normal children and young adults from the Pediatric Unit of Hospital de Clínicas of the UFPR, who blood sample withdrawn for routine laboratory analyses or preoperative analyses for elective surgeries. All individuals and/or guardians answered a questionnaire to evaluate the presence of signs and symptoms of IgAD, history of respiratory and gastrointestinal infection and duration of breast feeding. Informed consent was obtained from all guardians and patients. Eighty-five (57%) patients were male and 65 (43%) were female. The age range was 5.0 to 21.0 years. The mean age was 10.4 ± 3.3 years and the median age was 10.0 years.

Ethical approval was obtained from the Institutional Review Board (Ethics Committee) at Hospital de Clínicas of the UFPR.

The sera of patients were stored at -80 °C before determination of IgA concentration by three different methods. When serum IgA was not detected by turbidimetry patients were retested twice by RID. The RID plate (LC Partigen IgA, Behring), composed of...
agarose gel, contained monospecific antibodies of purified anti-chain α of human IgA. The limits for RID detection were 0.84 to 1.3 mg/dl.

Samples in which serum IgA levels remained undetectable by the two previous methods were tested by ELISA.

In patients with IgAD, the determination of IgG and IgM was performed by turbidimetry to exclude other humoral immunodeficiencies presenting lower IgA levels, such as hypogammaglobulinemia and common variable immunodeficiency. All results were expressed as mg/dl.

**Measuring IgA by ELISA method**

The ELISA to detect IgA was prepared and standardized in the Laboratory of Hospital de Clínicas of the UFPR and consisted of:

1. Sensitization of an ELISA Nune Maxisorp plate (Nalge Nune International Roskilde, Denmark), using anti-α chain of human IgA goat antibody (Sigma®, USA);
2. Addition of human IgA (Calbiochem, USA) or patient serum to create a pattern curve;
3. Incubation with the anti-α chain of peroxidase conjugated human IgA goat antibody (Sigma®, USA).

An ELISA plate was sensitized with anti-α chain of human IgA goat antibody at 10 µL/ml diluted in 0.05 M carbonate buffer at pH 9.6 (coating buffer) at 4 °C for at least 12 hours. The plate was then washed three times with a solution of 0.5% Tween 20 (v/v) in 0.15 M NaCl. Remaining protein-binding, sites were blocked for one hour at 37 °C by adding 100 µL of 2% casein (p/v) diluted in 0.05 M phosphate buffer and 0.15 M NaCl at pH 7.4.

After washing the plate, human IgA triplicates were added by serial dilution (3.9 to 500 ng/ml), the total sera of IgAD patients (n = 4) diluted to 1:20 (n=4) or the sera of normal patients (controls) diluted in 1:50.000 with 0.05 M phosphate buffer and 0.15 M NaCl at pH 7.4.

After one hour incubation at 37 °C and further washing, 100 µL of peroxidase conjugated anti-α chain human IgA goat antibody was added. The antibody was diluted to 1:16,000 in buffer solution.

After a final incubation and washing, 100 µL of revealed solution was added, composed of 0.02% o-phenylenediamine (p/v) dissolved in citrate-phosphate buffer containing 0.025 M citric acid, 0.05 M sodium phosphate at pH 5.0 and 30 volumes of hydrogen peroxide (0.02% v/v). After incubation for 15 minutes, the enzymatic reaction was interrupted by the addition of 20 µL of 2 M sulphuric acid.

The absorbance was determined at a wave length of 450 nm.

**RESULTS**

Normal serum IgA level, that is, over 5 mg/dl, was determined by ELISA in 150 patients of the Control Group and ranged from 44 to 509 mg/dl.

In the group of DM-1 patients, 141 out of 149 (94.6%) had normal levels of serum IgA detected by turbidimetry (Table 1). The turbidimetry method as well as RID, with the minimum limit of detection at 0.84 mg/dl, were ineffective to evaluate eight patients (8/149, 5.3%) whose serum IgA concentrations were below the detectable range.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DM-1 (n = 141)</th>
<th>Controls (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.7-22.5</td>
<td>5-21</td>
</tr>
<tr>
<td></td>
<td>12.6 ± 9.4*</td>
<td>10.4 ± 3.3*</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>77:72</td>
<td>85:65</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>7.0 ± 3.5*</td>
<td>-</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>5.4 ± 4.2*</td>
<td>-</td>
</tr>
<tr>
<td>Serum IgA (mg/dl)</td>
<td>51-476</td>
<td>44-509</td>
</tr>
</tbody>
</table>

The determination of serum IgA level in these eight patients was performed by ELISA considering its greater sensitivity over the two previous methods.

The ELISA showed that eight patients had serum IgA concentrations lower than 5 mg/dl, indicating IgAD (Chart 1). The presence of normal serum levels of IgG and IgM determined by turbidimetry confirmed the diagnosis of IgAD by ruling out other humoral immunodeficiciences.

<table>
<thead>
<tr>
<th>ELISA IgA</th>
<th>IgG</th>
<th>IgM</th>
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<tbody>
<tr>
<td>0.000078</td>
<td>2260</td>
<td>192</td>
</tr>
<tr>
<td>0.000096</td>
<td>3060</td>
<td>192</td>
</tr>
<tr>
<td>0.000027</td>
<td>2060</td>
<td>329</td>
</tr>
<tr>
<td>0.013427</td>
<td>2890</td>
<td>217</td>
</tr>
<tr>
<td>0.000079</td>
<td>3770</td>
<td>319</td>
</tr>
<tr>
<td>0.00058</td>
<td>1900</td>
<td>109</td>
</tr>
<tr>
<td>0.000358</td>
<td>2650</td>
<td>271</td>
</tr>
<tr>
<td>0.0014647</td>
<td>2490</td>
<td>261</td>
</tr>
</tbody>
</table>

The mean age of these eight patients was 14.6 ± 3.1 years. Their mean age upon diagnosis of DM-1 was 8.9 ± 3.4 years and the duration of the disease was 5.7 ± 4.9 years. The mean age of patients with IgAD, their mean age at diagnosis of DM-1 as well as duration of breast-feeding were not statistically different from the DM-1
Group. None of the IgAD patients reported recurrent respiratory or gastrointestinal infections.

**DISCUSSION**

According to the Latin American Group of Immunodeficiencies Latin American Registry of Primary Immunodeficiencies (LAGID), humoral immunodeficiencies account for 58% of cases of these disorders – IgAD is the most common. One clinical study performed in Brazil involved 11,576 healthy blood donor adults, including pregnant women, and found 12 IgAD patients – a prevalence of 1:965.

In the present study, IgAD was defined as serum IgA levels lower than 5 mg/dl, accompanied by normal or increased levels of serum immunoglobulin G (IgG) and M (IgM).

None of the patients in the Control Group (n = 150) had IgAD; however IgAD was detected in 5.3% of DM-1 patients. This figure is approximately 50-fold higher than the prevalence of IgAD in the general Brazilian population, which is estimated at 1:1000.

The prevalence of IgAD in the normal population in France is 1:1400, and Liblau et al. detected one IgAD patient in every 261 diabetics. A similar study was carried out in Italy, where the prevalence of IgAD in the normal population was found to be 1:500 and one patient with IgAD was detected for 27 diabetes patients.

In IgAD, serum IgA was detected by ELISA, a method that is more precise and sensitive than turbidimetry and RID. The confirmation of IgAD was necessary to avoid false-negative serological tests as it would occur in DM-1 patients with other autoimmune diseases. For example, CD screening is based on IgA antigliadin and IgA antientomysium antibodies. This study recommends the use of ELISA as a more sensitive method to determine serum IgA levels.

Very low levels of IgA were detected in four patients with IgAD corresponding to 200,000-fold lower than the lowest limit of IgA established by the WHO (5 mg/ml). The serum used for ELISA was diluted five times.

None of the eight IgAD patients had recurrent upper or lower respiratory infections or gastrointestinal infections. Most cases of IgAD are asymptomatic despite greater susceptibility to infections. The absence of symptoms among these patients could be explained by the presence of B cells expressed as surface IgM in gastrointestinal mucosa and the acquisition of a compensatory mechanism making IgM capable of transporting the secretory component across the epithelium.

Some other studies completed in Brazil showed different prevalence of IgAD in diabetes patients. In São Paulo, Baptista et al. screened diabetic patients for celiac disease and found that 3/104 (2.9%) of them had IgAD. In the northeastern region of Brazil, Araújo et al. found a prevalence of 2.0% of IgAD among diabetic patients. These findings indicate higher prevalence in DM-1 patients than in the normal Brazilian population (1:965).

**CONCLUSIONS**

Due to the high prevalence of IgAD among DM-1 patients (5.3%) detected in the present study, the measurement of serum IgA is recommended in all DM-1 patients.

**REFERENCES**


