High serum IgA concentrations in patients with diabetes mellitus: agewise distribution and relation to chronic complications

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In this study we investigated the agewise distributions of serum IgA concentrations in 1251 type 1 and 2224 type 2 diabetic patients, and the association between serum IgA concentration and diabetic complications (retinopathy, nephropathy, macroangiopathy, and hypertension). The IgA concentrations of all groups of diabetic patients were significantly higher than those of the corresponding subgroups of 943 control subjects, except for type 1 patients >60 years of age. High IgA concentrations were found in 23.1% of the whole diabetic group. The prevalence of high IgA was significantly greater in males than in females among type 1 patients (24.4% vs 18%). In conclusion, an increase in circulating IgA concentrations is a generalized phenomenon among diabetic patients; IgA concentrations above the reference range are more common among male than female diabetics; and diabetic complications are associated with a significant increase in serum IgA concentration.

INDEXING TERMS: diabetes subtypes • retinopathy • nephropathy • hypertension • macrovascular disease

In an earlier study [1], we found that immunoglobulin A (IgA) has a marked effect on fructosamine determinations: 80% of a group of nondiabetics with abnormally high IgA concentrations but no hepatic disease had abnormally high fructosamine concentrations. Subsequently [2], we found that some 30% of a group of 169 diabetic patients had high serum IgA concentrations, the same prevalence holding among both insulin-dependent (type 1) and non-insulin-dependent (type 2) diabetics; and in both subgroups, high serum IgA concentration was more common among patients who had been under treatment for 10 years or more than among more recent patients.

Singh and Kulig [3] published tacit corroboration of high serum IgA concentrations among diabetics: The mean IgA concentration in their group of 149 diabetic patients was 3.5 ± 1.6 g/L, ~70% higher than in 54 healthy subjects (2.1 ± 0.8 g/L) or 149 nondiabetic patients (2.3 ± 0.9 g/L), and the difference was maintained regardless of whether the diabetic patients had kidney disease (3.5 ± 1.6 g/L, n = 50), liver disease (3.7 ± 2.1 g/L, n = 15), both (3.6 ± 1.7 g/L, n = 65), or neither (3.4 ± 1.6 g/L, n = 84).

To confirm the association between diabetes and high serum IgA concentration, and bearing in mind the statistically significant differences among the serum IgA concentrations of healthy subjects of different age groups [4], in this study we determined the agewise distribution of circulating IgA concentration in broad samples of control subjects and diabetic patients of both types. We also studied whether high IgA concentrations were associated with microvascular and (or) macrovascular diabetic complications.

Materials and Methods

SUBJECTS
The diabetic outpatient clinics at the Galician General Hospital, Santiago de Compostela, Spain, are attended by almost all adult and pediatric type 1 diabetic patients in the district and by most type 2 patients requiring treatment with insulin or oral anti-diabetic medications. The sample studied in this work comprised 1251 type 1 patients and 2224 type 2 diabetic patients who attended our clinics at least three times between January 1, 1992, and December 31, 1994. Among other quantities, IgA was determined in all these patients.
Type 1 and type 2 diabetes mellitus were diagnosed in accordance with the criteria of the US National Diabetes Data Group [5]. For this study, patient age was defined as age at the time of the patient's latest analytical determination.

Reference ranges for serum IgA were established with sera from 943 healthy subjects who volunteered themselves or were volunteered by their parents; these subjects were recruited among hospital staff and their children, children attending playschools, and residents of old people's homes belonging to the same geographical area as the diabetic patients. The good health of all of these subjects was confirmed by medical examination at the time of blood sampling.

The approval of the Hospital Ethics Committee was obtained before initiation of the study.

**ANALYTICAL PROCEDURES**
Venous blood samples were taken from an antecubital vein. Serum IgA was determined in an Array Protein System Analyzer (Beckman Instruments, Fullerton, CA) by using appropriate antisera and controls. Twenty-four-hour urine was tested for proteinuria with Labstix dipsticks (Ames, Elkhart, IN); the total protein concentration in urine specimens for which dipstick testing showed any trace of protein was quantified by the trichloroacetic acid method [6].

**DIAGNOSTIC CRITERIA FOR ASSESSMENT OF CLINICAL COMPLICATIONS**

Retinopathy. Patients were considered to have retinopathy if they exhibited retinal microaneurysms, soft exudates, small intraretinal hemorrhages, venous bleeding, neovascularization, or retinal traction or detachment. The presence of retinopathy was determined by an experienced observer by direct ophthalmoscopy through pupils dilated with 5 g/L tropicamide.

Neuropathy. Vibration thresholds at both medial malleoli and both great toes were determined by using a biothesiometer (Biomedical Instrument Co., Newbury, OH); neuropathy was considered to be present if the thresholds were >2 above the mean of the control subjects of the same age group [7]. Neuropathy was also diagnosed when there were symptoms compatible with sensorimotor polyneuropathy, autonomic neuropathy or mononeuropathy, absent ankle reflexes, or impairment of light touch or pin-prick sensation in the feet.

Nephropathy. Patients were considered nephropathic if they exhibited proteinuria of 1 g/L (or 0.3 g/L on more than one occasion) in the absence of infection and of any evident cause of renal disease other than diabetes.

Macrovascular disease. Macrovascular disease was considered to be present if there was a history of myocardial infarction, angina, stroke, intermittent claudication, vascular surgery, or amputation for atherosclerotic disease, or if one or more foot pulses were absent.

Hypertension. Patients were defined as hypertensive if (a) they were on antihypertensive drugs, (b) their systolic blood pressure was >140 mmHg (160 mmHg for patients >60 years of age), or (c) their diastolic blood pressure was >90 mmHg (95 mmHg for patients >60 years of age); blood pressures used for classification in accordance with this definition were the means of two determinations.

**STATISTICAL ANALYSIS**
Since skew and kurtosis values showed that none of the distributions was gaussian, the median was used as a measure of location. Reference ranges for the following age groups were established as the interval between the 2.5% and 97.5% quantiles of the corresponding control sample: 1–10 years (61 subjects), 11–20 years (147), 21–30 years (220), 31–40 years (182), 41–50 years (132), 51–60 years (94), and >60 years (107). Patients were deemed to have high serum IgA concentrations when their serum IgA concentration exceeded the upper end of the reference range for their age group. For brevity in what follows, we will also use “range” to refer to the 2.5–97.5 percentile interval when applied to data for diabetic groups.

The computer package SPSS version 6.0 for Windows 3.1 (SPSS, Cary, NC) was used to process the data. The significance of differences between groups was estimated by Wilcoxon's test; P <0.05 was considered significant, and the Bonferroni criterion was used when multiple significance tests were made. The significance of differences between percentages was estimated by Pearson's χ² test.

**Results**

**SERUM IgA CONCENTRATIONS**
Table 1 lists the serum IgA concentrations (median and reference range; see Material and Methods) in each age group of the 943 control subjects, together with the median and range in each age group of the 1251 type 1 patients and the 2224 type 2 patients. Fig. 1 shows the frequency distributions of IgA concentrations among type 1 and type 2 diabetic patients.

The observed reference ranges are similar to those reported by other authors who used an analytical method similar to ours [4]. The overall median among the control subjects was 1.46 g/L (range 0.22–3.89 g/L); in no age group was there a statistically significant difference between male and female controls.

The overall median among type 1 patients was 2.01 g/L (range 0.29–7.01 g/L), and the overall median among type 2 patients was 2.67 g/L (range 0.67–9.86 g/L). These values differed significantly from the median for control subjects (P <0.005 for type 1, P <0.001 for type 2).

Type 1 and type 2 age groups had significantly higher IgA concentrations than the control groups, except for the type 1 patients ages <20 years and >60 years, and the type 2 patients ages <50 years, keeping in mind the Bonferroni criterion. Among type 1 patients the greatest difference occurred for the 41–50-year-old age group, which had a median IgA concentration 71% higher than that of the control group, whereas among type 2 patients the greatest difference occurred for the 51–60-year-old group, whose median IgA concentration was 65% higher than that of the control group.

High serum IgA concentrations were exhibited by 21.3% of type 1 diabetic patients and by 24.1% of type 2 patients. High
Table 1. Serum IgA concentrations (g/L) in control subjects and diabetic patients classified by age group.

<table>
<thead>
<tr>
<th>Age years</th>
<th>n</th>
<th>Median (ref. range)</th>
<th>Controls</th>
<th>n</th>
<th>Median (range)</th>
<th>P</th>
<th>Type 1 patients</th>
<th>n</th>
<th>Median (range)</th>
<th>P</th>
<th>Type 2 patients</th>
<th>n</th>
<th>Median (range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>61</td>
<td>0.67 (0.22-2.20)</td>
<td>206</td>
<td>85</td>
<td>0.99 (0.29-2.30)</td>
<td>&lt;0.05</td>
<td></td>
<td>70</td>
<td>1.90 (0.73-3.48)</td>
<td>&lt;0.05</td>
<td></td>
<td>1246</td>
<td>2.81 (0.67-9.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11-20</td>
<td>147</td>
<td>1.15 (0.45-2.80)</td>
<td>468</td>
<td>1.70 (0.42-5.50)</td>
<td>&lt;0.01</td>
<td></td>
<td>241</td>
<td>2.43 (0.74-4.90)</td>
<td>&lt;0.01</td>
<td></td>
<td>667</td>
<td>2.65 (0.71-6.40)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>220</td>
<td>1.48 (0.57-3.04)</td>
<td>283</td>
<td>2.20 (0.51-6.18)</td>
<td>&lt;0.005</td>
<td></td>
<td>667</td>
<td>2.65 (0.71-6.40)</td>
<td>&lt;0.005</td>
<td></td>
<td>1246</td>
<td>2.81 (0.67-9.86)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>182</td>
<td>1.50 (0.66-3.10)</td>
<td>256</td>
<td>2.33 (0.92-6.70)</td>
<td>&lt;0.005</td>
<td></td>
<td>70</td>
<td>1.90 (0.73-3.48)</td>
<td>&lt;0.05</td>
<td></td>
<td>1246</td>
<td>2.81 (0.67-9.86)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>132</td>
<td>1.57 (0.69-3.07)</td>
<td>96</td>
<td>2.69 (0.73-5.24)</td>
<td>&lt;0.001</td>
<td></td>
<td>241</td>
<td>2.43 (0.74-4.90)</td>
<td>&lt;0.01</td>
<td></td>
<td>667</td>
<td>2.65 (0.71-6.40)</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>94</td>
<td>1.61 (0.70-3.09)</td>
<td>49</td>
<td>2.34 (1.70-7.01)</td>
<td>&lt;0.001</td>
<td></td>
<td>667</td>
<td>2.65 (0.71-6.40)</td>
<td>&lt;0.005</td>
<td></td>
<td>1246</td>
<td>2.81 (0.67-9.86)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>107</td>
<td>1.95 (0.72-3.89)</td>
<td>14</td>
<td>1.93 (1.50-2.58)</td>
<td>NS</td>
<td></td>
<td>70</td>
<td>1.90 (0.73-3.48)</td>
<td>&lt;0.05</td>
<td></td>
<td>1246</td>
<td>2.81 (0.67-9.86)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significantly different when corrected for multiple testing, using Bonferroni adjustment.

Fig. 1. Frequency distributions of serum IgA concentrations in diabetic patients of types 1 (white bars) and 2 (shaded bars). Arrows indicate the upper limits of the reference ranges for subjects ages <60 years (3.1 g/L) and >=60 years (3.9 g/L).

IgA was significantly more common among males than among females for type 1 patients, among whom 24.4% of males and 18% of females had high IgA.

ASSOCIATION BETWEEN HIGH IGA AND DIABETIC COMPLICATIONS

Table 2 lists the serum IgA concentrations of various patient subgroups defined on the basis of their micro- or macrovascular diabetic complications, distinguishing in each case between type 1 and type 2 patients. For type 1 patients, all the groups with complications had significantly higher IgA concentrations than patients of the same type without complications, but for type 2 patients the IgA concentrations were significantly different in those with retinopathy and macroangiopathy, when corrected for multiple testing. Among type 1 patients, the group of patients with macroangiopathy had lower IgA concentrations than any of the other groups distinguished, whereas among type 2 patients the macroangiopathy group had the highest IgA concentrations.

Among type 1 patients, the neuropathy group had higher IgA concentrations than the other groups with microvascular complications, whereas among type 2 patients the retinopathy group had analogous status. In both cases, however, the differences with respect to other microvascular groups were minimal. Among both type 1 and type 2 patients, the group with all three microvascular complications had higher IgA concentrations than the group with just one complication.

The groups with the highest proportions of members with high IgA were the neuropathy group for type 1 patients (31.9%) and the macroangiopathy group for type 2 patients (27.3%).

Discussion

The results of this study of a rather large sample of diabetic patients confirm the high average serum IgA concentrations

Table 2. Serum IgA concentrations (g/L) in diabetic patients classified by clinical complications.

| Clinical complication | Type 1 patients | | | Type 2 patients | | |
|-----------------------|-----------------|-----------|-----------------|-----------------|-----------|-----------------|-----------|
| None                  | n   | Median (range) | P    | n   | Median (range) | P    |
| Retinopathy           | 471 | 2.29 (0.42-7.01)| <0.001| 1335 | 2.75 (0.67-10.7) | 0.002|
| Nephropathy           | 149 | 2.27 (0.61-6.84)| <0.001| 369 | 2.73 (0.71-10.7) | 0.005|
| Neuropathy            | 442 | 2.36 (0.47-7.01)| <0.001| 1337 | 2.67 (0.69-10.7) | 0.045|
| Retinopathy, nephropathy, or neuropathy | 252 | 2.31 (0.61-4.88)| <0.001| 934 | 2.62 (0.69-10.0) | 0.028|
| Retinopathy, nephropathy, and neuropathy | 126 | 2.39 (0.61-7.01)| <0.001| 269 | 2.73 (0.71-10.7) | 0.016|
| Hypertension          | 115 | 2.30 (0.42-7.01)| <0.001| 848 | 2.72 (0.67-10.7) | 0.006|
| Macroangiography      | 91  | 2.01 (1.13-7.01)| <0.001| 750 | 2.79 (0.67-10.7) | 0.001|

* With respect to patients of the same type without complications.

* Statistically significantly different when corrected for multiple testing, using Bonferroni adjustment.
among such patients, in keeping with previous reports based on smaller samples [2, 3]. The overall median serum IgA concentration in the whole sample of 3475 patients was 2.43 g/L, 66% higher than the median for the 943 controls, 1.46 g/L. The difference is similar to the 70% increase (3.5 g/L vs 2.1 g/L for controls) reported by Singh and Kulig [3] (the difference in absolute values between their results and ours may be ascribed to their having used a different method for determining serum IgA). The increase affected both type 1 and type 2 patients (although more for the latter: 2.67 g/L vs 2.01 g/L, P < 0.01), and all age groups except the group of type 1 patients >60 years of age.

Some 23% of individual patients had high IgA, defined as a concentration above the reference range for the individual's age group; the figures for type 1 and type 2 patients separately were similar, 21.3% and 24.1%, respectively. High IgA was more prevalent among males than females, especially for type 1 diabetics, among whom 45% more males than females had high IgA (vs a 14% difference among type 2 diabetics).

All the type 1 and type 2 subgroups defined on the basis of their micro- or macrovascular diabetic complications had higher serum IgA concentrations than the corresponding groups of patients without complications. This suggests that monitoring IgA may provide early warning of the possible presence of complications. Furthermore, among both type 1 and type 2 patients, patients with all three kinds of microangiopathy had slightly higher IgA concentrations than patients with only one kind. However, for neither type 1 nor type 2 patients were there any significant differences among the IgA concentrations of the various groups with complications, except that, for both types, the group of patients with both nephropathy and hypertension had higher IgA concentrations than the other groups (and also a high prevalence of high IgA, 38%). The reason why the differences between the subgroups with microvascular complications or hypertension and the subgroup with no complications were greater for type 1 than for type 2 patients was apparently that the type 2 group with no complications had significantly higher IgA concentrations than the type 1 group with no complications (P < 0.001). The macroangiopathy group had the lowest IgA concentrations among the type 1 subgroups with complications, and the highest among the type 2 subgroups.

Koschinsky et al. [8] found that self-antibodies of all immunoglobulin classes against the specific epitope 4-furanoyl-2-furoyl-1H-imidazole-1-hexanoic acid (FFI) are common in both healthy and diabetic persons, but that FFI-specific IgA differed from FFI-specific IgG and IgM in that its mean titer in diabetic sera was significantly higher than its mean titer in healthy sera. This suggests that high IgA concentrations in diabetic patients are the result of an immune response to advanced glycosylation end products. It also suggests that the prevalence of high IgA among diabetics might be considerably higher than the overall 23% found in this study if "high IgA" is defined in terms of some relevant specific IgA or IgA subclass rather than as total IgA.

In conclusion, this study confirms that increased concentrations of circulating IgA are general among both type 1 and type 2 diabetic patients, that this phenomenon is more marked among males than among females, and that high serum IgA is a nonspecific sign of the development of diabetic complications.

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References