Longitudinal study of fasting proinsulin in 148 siblings of patients with insulin-dependent diabetes mellitus

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Abstract

Objective: To follow proinsulin immunoreactive material (PIM) in healthy siblings from the time of diagnosis of insulin-dependent diabetes mellitus (IDDM) in the proband, for at least 2 years.

Design and methods: The study comprised 148 siblings representing 112 families. The siblings were recruited from the nationwide ‘Childhood Diabetes in Finland’ study and tested for immunological markers. If a sibling was found positive for islet cell antibodies (ICA) or insulin autoantibodies (IAA), PIM sampling was extended beyond 2 years.

Results: Of the 148 siblings, 12 developed IDDM 3–53 months after the diagnosis in the proband. Eleven of these siblings exhibited initially normal PIM concentrations. In nine siblings, samples were available both more than 6 months and during the last 6 months before the diagnosis of IDDM; PIM concentrations increased in seven, remained unchanged in one, and decreased in one in the period up to the diagnosis of IDDM \( (P < 0.05) \). Median PIM concentration did not change significantly during the examination period of 2 years in the 136 siblings who did not contract IDDM. Constantly increased PIM concentrations were found in 12 of the 136 siblings who did not develop IDDM. These 12 siblings were all ICA negative.

Conclusion: In healthy siblings of IDDM patients exhibiting an initially low PIM concentration, an abrupt increase in PIM seems to precede the clinical manifestation of IDDM within 0–6 months. However, there were too few patients available to close follow-up to allow calculation of any predictive value of this increase. Persistently increased PIM concentrations were present in some healthy siblings who did not develop IDDM. The reason for that finding remains unclear, but it could be associated with previous B cell damage.

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Introduction

In a cross-sectional study of healthy siblings of patients with insulin-dependent diabetes mellitus (IDDM), we found increased fasting proinsulin immunoreactive material (PIM) in 30% of the group, despite normal blood glucose and fasting insulin concentrations (1). Similar results have been found in identical twins, discordant for IDDM for more than 11 years (2). The significance of increased PIM concentrations, PIM/insulin or PIM/C-peptide ratios in these healthy relatives of patients with IDDM is still unclear. It cannot be explained by a simple hyperactivity of the B cells, because both fasting and stimulated blood glucose concentrations were normal in these individuals (1–3). Furthermore, it is well documented that the PIM/insulin (PIM/C-peptide) ratio is not increased in the healthy obese (4–6) who have an increased demand for insulin. In these people, both PIM and insulin (C-peptide) are proportionally increased, leaving the ratio unchanged. Also, in lean healthy subjects B cell stimulation by exogenous glucose does not result in a changed PIM/insulin (C-peptide) ratio (7, 8). The increased PIM in healthy siblings of patients with IDDM could be an indicator for risk of progression to IDDM, but this is unlikely, as the twins and siblings (1, 2) were discordant for several years, and there was no difference in PIM concentrations between HLA-identical, haplo-identical and non-identical siblings (1). Islet cell antibodies (ICA) have been observed to be associated with increased PIM concentrations (9), but most of the first-degree relatives in previous studies tested negative for ICA (1, 2, 9). However, in some siblings we found increased PIM concentrations or PIM/C-peptide ratios associated with decreased B cell function and later progression to IDDM (10).

In an attempt to clarify these issues further, we studied PIM concentrations longitudinally in a
Table 1 Clinical characteristics of initially unaffected siblings of patients with IDDM, at the time of entry to the study. Values for the siblings developing IDDM are median and (range); those for siblings remaining unaffected are median and (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Siblings developing IDDM (n = 12)</th>
<th>Siblings remaining unaffected (n = 136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>3/9</td>
<td>51/85</td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.7 (2.6–11.6)*</td>
<td>10.8 (5.7–14.5)</td>
</tr>
<tr>
<td>F-BG (mmol/l)</td>
<td>4.5 (3.9–5.1)</td>
<td>4.6 (4.2–4.9)</td>
</tr>
<tr>
<td>F-PIM (pmol/l)</td>
<td>4.0 (3.9–5.1)</td>
<td>4.0 (1.7–8.7)</td>
</tr>
<tr>
<td>F-CP (pmol/l)</td>
<td>500 (190–1130)</td>
<td>360 (250–550)</td>
</tr>
<tr>
<td>F-PIM/CP (%)</td>
<td>0.9 (0.3–2.0)</td>
<td>1.3 (0.7–2.3)</td>
</tr>
</tbody>
</table>

F, fasting; BG, blood glucose; PIM, proinsulin immunoreactive material; CP, C-peptide. *P < 0.05, between groups.

Research design and methods

From the nationwide ‘Childhood Diabetes in Finland (DiMe) study’, 148 siblings of patients with IDDM were selected for this survey on the basis of availability of consecutive blood samples obtained in the fasting state. The siblings derived from a population of siblings (n = 765) of newly diagnosed children with IDDM (11, 12). Clinical data of the siblings developing IDDM and of those remaining unaffected throughout the period of observation are presented in Table 1.

Fasting PIM concentrations were measured in the healthy sibling from the time of diagnosis in the proband, at 2 weeks and at 2–3, 5–6, 11–12, 17–18 and 23–24 months. If a sibling was found positive for either ICA or insulin autoantibodies (IAA), sampling was extended beyond 2 years. In some of these siblings, blood samples were available at intervals of less than 3 months up to the diagnosis of IDDM.

Fasting blood glucose concentrations were measured initially and at 24 months. Fasting C-peptide concentrations were measured when the participant entered the study, but then only at various intervals during the subsequent 2 years.

The study was approved by the local ethics committees in Finland, and all participants, their parents, or both, gave informed consent.

Methods

PIM concentrations were measured by a two-site enzyme-linked immunosorbent assay (13). Intra- and interassay coefficients of variation (CV) were 8.0% and 9.8% respectively. The limit of detection of PIM in serum was 1.8 pmol/l; values less than this were listed as 1.7 pmol/l. From the assay evaluation and former studies of adolescents, 13 pmol/l was used as the upper limit of normal (1, 13, 14). Using intact proinsulin as calibrator (100%), the four proinsulin conversion intermediates split(32–33), des(31,32), split(65–66), and des(64,65)-proinsulin had molar cross-reactivities throughout the measuring range of 65, 75, 90 and 95%, respectively. Serum C-peptide was measured by RIA (15) using antibody K6 (Novo Nordisk A/S, Bagsvaerd, Denmark). Intra- and interassay CV were 1.8% and 10% respectively; the limit of detection was 20 pmol/l.

Blood glucose was measured by the glucose oxidase method (16). ICA was determined by an immunofluorescence method from sections of frozen human group O pancreas (17) and IAA by a modified liquid phase RIA (18). Both the ICA and the IAA methods have been described in detail elsewhere (12). The limit of detection for the conventional indirect ICA method was 2.5 Juvenile Diabetes Foundation units (JDF-U); IAA was considered positive if insulin binding exceeded 2.4%, representing the mean + 3 S.D. in 100 non-diabetic individuals.

Statistics

All results are presented as medians and percentiles, unless stated otherwise. Comparisons over time in individual subjects were analysed with the Pratt matched-pair signed rank test. Mann–Whitney rank sum test was used to compare siblings remaining unaffected and siblings developing IDDM.

P < 0.05 was considered statistically significant (two-tailed).

Results

During follow-up, 12 siblings progressed to clinical IDDM. In those remaining unaffected, median PIM remained almost constant during the 2 years (Fig. 1, panel A). Median values at 2 weeks, 2–3, 5–6, 11–12, 17–18 and 23–24 months were 4.0, 5.1, 5.1, 6.8, 5.4, and 4.8 pmol/l respectively, with considerable between-individual variation. In contrast, the within-individual variations over time were small, some siblings constantly displaying high concentrations of PIM, others constantly normal values. To illustrate this, results from five measurements in each of two siblings are shown in Fig. 1, panel B. Twelve of the 136 unaffected siblings (8.8%) constantly had PIM concentrations greater than 13 pmol/l during the 2 years of examination. Siblings who were brothers or sisters younger or older than the proband had comparable values of blood glucose, C-peptide, PIM or PIM/C-peptide ratio.

The 12 siblings who developed IDDM were younger than the remaining 136 siblings (Table 1), but had indistinguishable values for blood glucose, C-peptide and PIM, and a similar sex ratio. IDDM was diagnosed from 3 to 53 months after IDDM diagnosis in the proband. At entrance to the study, i.e. at the time of diagnosis of IDDM in the proband, 11 had PIM concentrations less than 13 pmol/l and in one the
PIM concentration was 35 pmol/l. Samples from that sibling were unfortunately not available close to the diagnosis of IDDM. Table 2 displays the individual median PIM concentration from measurements more than 6 months before the diagnosis of IDDM, together with the median PIM concentrations from the last 6 months before diagnosis, but not including the value at the time of IDDM diagnosis. One sibling (No. 12) gave a sample only at 3 months before the diagnosis of IDDM; two gave samples a long time before diagnosis, but subsequently only at the time of diagnosis (Nos 1, 4). In the nine siblings with available samples, seven had increasing, one decreasing, and one unchanged PIM concentrations; the difference between PIM(0–6 months) and PIM(>6 months) was significant ($P < 0.05$; Table 2).

Among the 12 siblings who developed IDDM, one had no available IAA or ICA samples before IDDM was diagnosed.

**Table 2** Individual median PIM (pmol/l) over the last 6 months (−6 to 0 months) before the diagnosis of IDDM (not including values at the time of diagnosis) and the median PIM from the preceding period (x to −6 months; x representing values between −53 and −12 months) in the 12 siblings who developed IDDM.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>x to −6</td>
<td>5.8</td>
<td>6.2</td>
<td>5.3</td>
<td>18.0</td>
<td>1.7</td>
<td>14.0</td>
<td>11.0</td>
<td>2.2</td>
<td>4.8</td>
<td>7.5</td>
<td>4.1</td>
<td>—</td>
</tr>
<tr>
<td>−6 to 0</td>
<td>—</td>
<td>20.0</td>
<td>8.8</td>
<td>—</td>
<td>1.7</td>
<td>21.0</td>
<td>13.0</td>
<td>3.2</td>
<td>30.0</td>
<td>5.8</td>
<td>20.0</td>
<td>19.8</td>
</tr>
</tbody>
</table>

—, No sample.
discussed. Among the remaining 11 siblings, ICA was positive, the latest value before diagnosis of diabetes being 160 (10–1280) JDF-U (median and range). In three siblings, IAA was also positive (2.5, 2.8 and 6.4%). At entry to the study, after 1 year and at 2 years respectively, ICA was positive in six, six and seven of the 136 siblings remaining unaffected, values being in the ranges 10–80, 20–160, 10–320 JDF-U. All the 12 siblings with constantly high PIM concentrations and who remained well were ICA negative.

Discussion

The demonstration of increased PIM in first-degree relatives of patients with IDDM in the fasting state (1, 2, 9) and after stimulation (3), led initially to speculation of a dysfunction of the B cells (2, 19). That the B cell function is altered in some relatives of patients with IDDM is certain, but it remains unclear if an increased PIM concentration found in these relatives represents a dysfunction of the B cells or is related to later progression to IDDM. In two earlier studies, the siblings were not prone to develop diabetes, as they had been discordant for more than 5 years (1, 2). In fact, those siblings who later developed diabetes all had normal fasting PIM values (1). In the present study, all the siblings had been discordant only for a few weeks up to 2 years and no differences were found between the siblings who later developed IDDM and those who remained unaffected. A consistent finding was made in the cross-sectional survey by Heaton et al. (19), who studied PIM concentrations in non-diabetic identical twins of patients with IDDM diagnosed within 5 years (mean of 18 months). The twins were grouped according to the presence of ICA. Fasting blood glucose concentrations were normal, but the group with ICA had increased glucose concentrations after both oral and i.v. glucose load. Despite this, PIM concentrations were equally increased in twins with ICA and in twins without these antibodies. Combining these observations with their findings in the study with long-term discordance for IDDM (2), the authors concluded that the changes in B cell function, characterised by increased fasting PIM concentrations, do not necessarily progress to diabetes (19). However, we have previously found greater PIM concentrations in relatives with ICA, even though also the ICA-negative relatives had increased PIM concentrations compared with those in unrelated controls (9). The fact that HLA sharing is not associated with increased PIM concentrations speaks against a dysfunction of the B cells that would progress to IDDM in siblings with increased concentrations of PIM (1, 3).

In contrast, in some situations increased PIM concentrations were associated with a well established dysfunction of the B cells, namely a decreased first-phase insulin response (10). In the prediabetic phase of non-IDDM (NIDDM), controversial results have been reported, also. Recently, two studies have found increased PIM concentrations in individuals in the pre-NIDDM phase of the disorder, at a time when fasting blood glucose values were poorly discriminatory (20, 21). Other studies failed to demonstrate increased PIM concentrations in first-degree relatives of patients with NIDDM in two cross-sectional surveys, one comprising monozygotic twins (22, 23). Even though discordant monozygotic twins are at risk of later presenting with NIDDM, the exact number of progressors was not known in these series.

In first-degree relatives of patients with IDDM, increased fasting PIM appears to be found in two distinct groups, both illustrated in this survey. The first group comprises siblings with increased PIM who remained unaffected and in the long term seem less likely to develop diabetes. The second group includes siblings of patients with IDDM, who have increased PIM shortly before they themselves develop diabetes. Regarding the first group, we have earlier proposed that the increased PIM concentration could represent a genetically determined trait characterised by more vulnerable B cells, or may reflect previous B cell damage, which in the proband led to IDDM, but in the healthy sibling merely produced an altered B cell function but probably not a progressive loss of B cells. The findings by Lindgren et al. (24) of comparable PIM concentrations in newborn infants having a sibling with IDDM and in newborn infants with a negative family history for IDDM argue against the concept of a family trait, but dissimilarities emerging later in life cannot be excluded. Both groups of newborn infants had high PIM concentrations, confirming previous results (25) and probably reflecting immature B cells. In the present study, we followed PIM longitudinally in first-degree relatives and found no change over time in the siblings who remained unaffected. As in earlier cross-sectional studies, a proportion of the siblings had PIM concentrations above normal fasting values and continuously displayed this increased PIM concentration. This finding could support the theory of one or more previous B cell attacks, leaving the B cells with a slightly altered function, but this cannot be proven with the data available.

The second group with increased PIM concentrations were the 12 siblings who developed IDDM. In 11 of them, normal fasting PIM concentrations were found initially, and PIM increased abruptly a few months before the diagnosis of IDDM. To our knowledge, this is a new observation. Previously, high PIM concentrations have been found in patients with newly diagnosed IDDM (26, 27). The influence of PIM bound to insulin antibodies on the measured circulating concentration of PIM should be borne in mind (28). However, among the siblings in the present study, IAA was positive in only three, and the IAA values did not change close to the diagnosis of IDDM and therefore cannot explain the abrupt increase. At the time of the increase in PIM, the
siblings were ICA positive and most of them had low first-phase insulin response to an i.v. glucose tolerance test (10). However, ICA was in most cases present for a long time before the PIM concentrations increased abruptly. Even though fasting blood glucose values were normal, minor changes in blood glucose concentrations cannot, of course, be excluded; however, if that should be the mechanism for the increase in PIM, a strong correlation between fasting blood glucose and PIM concentrations would be expected. This was not the case. The size of the present series was too limited to allow assessment of the prediction of risk. However, if these new observations of abruptly increasing PIM concentrations preceding the clinical onset of IDDM are confirmed in larger series, this may prove to be a valuable predictive marker for IDDM in siblings who are at risk.

In conclusion, an abrupt increase in PIM seems to take place shortly before clinical onset of diabetes. This increase must be associated with B cell dysfunction. The change in PIM concentrations could be a marker (together with immunological markers) for later progression to IDDM. In contrast, constantly high fasting PIM concentrations over a long time seem not to be related to increased risk of developing IDDM.

References


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