THYROID, GASTRIC AND ADRENAL AUTO-IMMUNITY IN DIABETES MELLITUS

By

Jørn Nerup and Christian Binder

ABSTRACT

The clinical association between diabetes mellitus and auto-immune diseases of the thyroid, the adrenals and the gastric mucosa occurs more frequently than could be expected by chance. Sera from 133 patients with diabetes mellitus and 128 controls were therefore investigated for the presence of organ-specific auto-antibodies. Thyroid microsomal antibody and gastric-parietal-cell antibody were demonstrated with significantly increased frequency – 20% and 16% respectively – in sera from patients with diabetes mellitus. Antibodies reacting specifically with tissue components of the endocrine pancreas could not be demonstrated. From the data presented and from a review of the literature it is concluded that evidence is accumulating pointing to a disorder of the immunological system in patients with diabetes mellitus with regard to the formation of organ-specific humoral and cellular auto-immunity, and the occurrence of organ-specific auto-immune diseases.

During the last decade a few reports on thyroid and gastric auto-immunity in diabetes mellitus have been published. The literature was recently reviewed by Irvine et al. (1970), who themselves reported a significantly increased occurrence of thyroid microsomal and gastric-parietal-cell antibodies in sera from patients with diabetes mellitus.

The purpose of the present work was to study the incidence of auto-antibodies (thyroid microsomal, thyroglobulin, gastric-parietal-cell, adrenal and
The grouping of the 133 patients with diabetes mellitus according to sex, age, time of onset and duration of their disease: Group I includes all patients aged below 30 at the time of diagnosis. Group II comprises all patients aged above 50 at the time of diagnosis. Groups III and IV comprise patients with a duration of disease of less than one year and more than 10 years respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>39</td>
<td>27</td>
<td>66</td>
</tr>
<tr>
<td>Group II</td>
<td>23</td>
<td>35</td>
<td>58</td>
</tr>
<tr>
<td>Group III</td>
<td>25</td>
<td>17</td>
<td>42</td>
</tr>
<tr>
<td>Group IV</td>
<td>24</td>
<td>20</td>
<td>44</td>
</tr>
</tbody>
</table>

salivary duct antibodies and antinuclear factor) in sera from patients with diabetes mellitus, correlated with the duration and type of the disease.

**MATERIAL AND METHODS**

The material comprised sera from 133 patients with diabetes mellitus, taken consecutively among the patients admitted to the clinic, according to the criteria listed in Table 1.

The patients were matched according to age and sex with 128 control patients and healthy subjects without any history of endocrine or immunological disorder.

Microsomal thyroid antibody was demonstrated by an immunofluorescence technique as described by Holborrow et al. (1959). Thyroglobulin antibody was measured by means of thyroglobulin sensitized sheep red cells obtained from Burroughs Welcome & Co. as described by Fulthorpe et al. (1961).

Antibodies against gastric-parietal-cell cytoplasm, adrenal cortex and salivary duct cytoplasm were demonstrated by immunofluorescence techniques, using unfixed sections of human gastric mucosa, salivary glands and adrenals from the monkey (cercopithecus aethiops) and man as previously described by Irvine (1963), Bertram & Halberg (1964) and Blizzard et al. (1962).

An attempt to demonstrate antibody against the secretory cells of the islets of Langerhans were made by means of an indirect immunofluorescence technique. Normal pancreas from the rabbit, monkey and man was used as antigen.

Statistical evaluation was carried out by calculating

$$x^2 = \frac{(ad - bc - 1/2N)^2 \times N}{N_1 \times N_2 \times N_a \times N_b}$$

where a, b, c & d are the observed frequencies in a 2 x 2 contingency table and N₁, N₂, Nₐ & N₆ are the marginal totals of this table. The formula uses Yates’ correction for small samples.
Attempts to demonstrate a circulating antibody against secretory cells in the islets of Langerhans by means of the technique described were unsuccessful. The presence of organ-specific antibodies in the sera from patients and controls is shown in Table 2.

Antibodies to thyroid cytoplasm (thyroid microsomal antibody) and gastric-parietal-cell cytoplasm were the only auto-antibodies which were found to occur with significantly increased frequency in the sera of patients with diabetes mellitus - 20% and 16% respectively ($P < 0.001$ and $P < 0.025$).

The thyroid microsomal antibody was found with significant increased frequency in both male and female patients, while the gastric-parietal-cell antibody could be demonstrated with significant increased frequency in male patients only.

Thyroglobulin antibody was demonstrated with equal frequency in sera of patients and controls, but high titers, i.e. titers $> 2.500$ were found in the sera from eight patients as compared to one control subject.

Adrenal antibody was found in only three sera, all of them from female patients with insulin dependent diabetes.

Salivary duct antibody and anti-nuclear factor was found equally frequent in patients and control subjects.

The simultaneous occurrence of two or more antibodies to thyroid, adrenal

**Table 2.**

Incidence of different types of antibodies in the sera of 133 patients with diabetes mellitus as compared to the incidence found in controls, matched with respect to sex and age. Statistical evaluation was carried out by calculating $x^2$ applying Yates’ correction for small samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Diabetics</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 68</td>
<td>Female n = 65</td>
</tr>
<tr>
<td>Thyroid microsomal</td>
<td>12$^{2)}$</td>
<td>15$^{3)}$</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Gastric-parietal-cell</td>
<td>8</td>
<td>13$^{3)}$</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Salivary duct</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Antinuclear factor</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

$^{1)}$ $P < 0.001$  $^{2)}$ $P < 0.005$  $^{3)}$ $P < 0.025$  $^{4)}$ $P < 0.05$
Table 3.
Incidence of gastric-parietal-cell and microsomal thyroid antibodies in the sera of 66 patients with diabetes mellitus aged below 30 years at the time of onset of disease (juvenile diabetes) as compared to the incidence found in matched controls. Statistical evaluation was carried out by calculating $x^2$ applying Yates' correction for small samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Diabetics</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 39)</td>
<td>Female (n = 27)</td>
<td>Total (n = 66)</td>
<td>Male (n = 39)</td>
</tr>
<tr>
<td>Thyroid microsomal</td>
<td>5</td>
<td>6</td>
<td>11 (17 %)¹</td>
<td>1</td>
</tr>
<tr>
<td>Gastric-parietal-cell</td>
<td>5</td>
<td>8</td>
<td>13 (18 %)²</td>
<td>2</td>
</tr>
</tbody>
</table>

¹) $P < 0.05$

No differences between the two sexes were demonstrable.

cortex or gastric-parietal-cell cytoplasm was seen only in the sera of patients with diabetes mellitus.

Table 3 shows the occurrence of thyroid microsomal and gastric-parietal-cell antibody in the sera of patients aged below thirty at the time of diagnosis (Group I). The occurrence of these two antibodies was found to be significantly increased (thyroid microsomal antibody 17 % and gastric-parietal-cell antibody 18 %, as compared to 3 % and 6 % in the matched control series respectively). No differences between the two sexes were demonstrable.

Table 4.
Incidence of gastric-parietal-cell and microsomal thyroid antibodies in the sera of 58 patients with diabetes mellitus aged above 50 at the time of onset of the disease (maturity onset diabetes) as compared to the incidence found in controls, matched with respect to sex and age. Statistical evaluation was carried out by calculating $x^2$ applying Yates' correction for small samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Diabetics</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 23)</td>
<td>Female (n = 35)</td>
<td>Total (n = 58)</td>
<td>Male (n = 20)</td>
</tr>
<tr>
<td>Thyroid microsomal</td>
<td>5</td>
<td>9</td>
<td>14 (24 %)¹</td>
<td>0</td>
</tr>
<tr>
<td>Gastric-parietal-cell</td>
<td>2</td>
<td>5</td>
<td>7 (12 %)²</td>
<td>1</td>
</tr>
</tbody>
</table>

¹) $P < 0.01$
Table 5.
Incidence of gastric-parietal-cell and microsomal thyroid antibodies in the sera of 42 patients with diabetes mellitus with a duration of the disease of less than 1 year, as compared to the incidence found in controls matched with respect to sex and age. Statistical evaluation was carried out by calculating $x^2$ applying Yates’ correction for small samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Diabetics</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 25</td>
<td>Female n = 17</td>
</tr>
<tr>
<td>Thyroid microsomal</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Gastric-parietal-cell</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

1) $P < 0.05$

In the sera of patients in group II (maturity onset diabetes) the thyroid microsomal antibody was the only one found to occur more frequently than in the sera of the matched controls (24% and 4% respectively). The gastric-parietal-cell antibody was detected in 12% of sera of the patients and in 6% of the control sera. This difference was not significant (Table 4).

When patients in group I and II (juvenile diabetes versus maturity onset diabetes) were compared, the thyroid microsomal and the gastric-parietal-cell antibodies occurred with equal frequency in the two series.

Table 6.
Incidence of gastric-parietal-cell and microsomal thyroid antibodies in the sera of patients with diabetes mellitus with a duration of the disease of more than 10 years, as compared to the incidence found in controls matched with respect to sex and age. Statistical evaluation was carried out by calculating $x^2$ applying Yates’ correction for small samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Diabetics</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 24</td>
<td>Female n = 20</td>
</tr>
<tr>
<td>Thyroid microsomal</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gastric-parietal-cell</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

1) $P < 0.05$
Table 5 shows that sera of patients with diabetes mellitus of a duration of less than one year (group III) contained the thyroid microsomal antibody more often than the control sera, though the difference was significant only when male patients were considered. The gastric-parietal-cell antibody occurred equally frequent in the sera of patients of this group of diabetics and in the control sera.

In the sera of patients with long-standing diabetes (group IV) the thyroid microsomal as well as the gastric-parietal-cell antibody could be demonstrated statistically more frequently than in the sera of matched controls (Table 6).

No differences were demonstrable when the occurrence of thyroid microsomal and gastric-parietal-cell antibodies were compared between patients with newly diagnosed and those with long-standing diabetes mellitus (group III versus group IV).

**DISCUSSION**

The present results are in accordance with the findings of Irvine et al. (1970) as far as the prevalence in the sera of patients with diabetes mellitus of thyroid microsomal and gastric-parietal-cell antibody is concerned.

In this series the thyroid microsomal antibody and the gastric-parietal-cell antibody were found to occur with significantly increased frequency in the sera of the diabetes population.

The thyroid microsomal antibody occurred with increased frequency in the sera of diabetics, independent of age, sex or type of diabetes mellitus. The gastric-parietal-cell antibody, however, could not be demonstrated to be present with increased frequency in the sera of patients with maturity onset diabetes.

Tables 5 and 6 show, that the incidence of thyroid microsomal and gastric-parietal-cell antibody increases with increasing duration of the disease. These findings suggest, that the presence of organ-specific antibodies in the sera of patients with diabetes mellitus is a phenomenon not only correlated with the existence of the disease, but also related to the duration of the abnormal metabolic condition.

Thyroglobulin antibody was found more often in the sera of patients with diabetes mellitus than in the control sera, but the difference was not significant. It is, however, interesting to note that high titers (> 2,500) were found almost exclusively, in the sera of patients with diabetes mellitus.

Antibody to components of adrenocortical cell cytoplasm is regularly found in the sera of patients with idiopathic Addison's disease, but is extremely rare in other conditions (Nerup et al. 1966; Irvine et al. 1967). It is therefore worthy of note that a circulating antibody against adrenocortical cell cytoplasm was detected in three insulin dependent patients of our series. Organ-specific, anti-
adrenal cellular hypersensitivity has also been demonstrated to occur in conjunc-
tion with diabetes mellitus (Nerup & Bendixen 1969).

It is well established (Goudie et al. 1959; Bastenie et al. 1967; Halberg et al.
1968) that the occurrence of thyroid microsomal antibody and thyroglobulin
antibody in titers ≥ 250 in the sera of patients without overt thyroid disease
reflects the presence in the thyroid gland of a focal or diffuse thyroiditis.

Ungar et al. (1968) found a prevalence of latent pernicious anaemia in
patients with diabetes mellitus to be increased and Irvine et al. (1970) demon-
strated, that a surprisingly high number of patients with diabetes mellitus had
intrinsic-factor antibody in their serum and achlorhydria. This is interesting in
view of the general finding, that the occurrence of intrinsic-factor antibody in
the serum shows a high correlation with advanced atrophic gastritis (te Velde
et al. 1964; Irvine 1965; Ardenman et al. 1966).

Consequently subclinical, functional and pathological damages to the thyroid
gland, the gastric mucosa and perhaps the adrenal cortex, parallel to the
immunological findings might be anticipated in diabetes mellitus.

The clinical association between diabetes mellitus and auto-immune diseases
has been reported to occur more frequently than would be expected by chance.
The auto-immune diseases in question are pernicious anaemia (Arapakis et al.
1963; Witts 1963; Munichoodapppa & Kozak 1970), myxoedema and chronic
thyroiditis (Asfeldt & Bretlau 1964; Drube & Seusing 1965; Hecht & Gershberg
1968) and idiopathic Addison’s disease (Solomon et al. 1965; Irvine et al. 1967;
Turkington & Lebovitz 1967).

Attempts to demonstrate circulating antibodies in the sera of patients with
diabetes mellitus reacting specifically with tissue components of the islets of
Langerhans have so far been unsuccessful (Irvine 1969, personal communica-
tion) and this, however, was not achieved during our work. Although anti-
pancreatic cellular hypersensitivity was recently demonstrated in patients with
diabetes (Nerup et al. 1971) the possibility that diabetes mellitus in some in-
stances could be a disease of auto-immune character is still not proved.

Thus evidence is accumulating, pointing to a disorder of the immunological
system in patients with diabetes mellitus with regard to the formation of
organ-specific humoral and cellular auto-immunity and the occurrence of
organ-specific auto-immune diseases.

REFERENCES


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