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# Seasonality of Type 1 (insulin-dependent) diabetes mellitus: values of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ show evidence of a more rapid loss of insulin secretion in epidemic patients

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**Summary.** According to month of diagnosis, 165 children who developed Type 1 (insulin-dependent) diabetes mellitus at the age of 0–16.2 years (mean ± SD, 7.6 ± 4.1 years) could be divided into 69 patients diagnosed during peak seasons (epidemic cases) and 96 patients diagnosed during months of low incidence (non-epidemic cases). Seasonality of onset of symptoms and of diagnosis was observed in both sexes in all age groups. The patients diagnosed during peak seasons had shorter duration of symptoms (13.2 ± 8.1 days) as compared to 22.9 ± 10.3 days; $p < 0.001$ in the patients diagnosed during months of low incidence. At diagnosis, 88.4% (61/69) of the epidemic group had ketonuria as compared to 71.9% (69/96); $p < 0.06$ in the non-epidemic patients. The values of C-peptide, insulin antibodies, haemoglobin $A_{1c}$ and HLA-DR phenotype frequencies in the 69 epidemic patients were compared with those of the 96 non-epidemic patients. In the epidemic patients, the C-peptide values of 0.11 ± 0.05 mmol/l at diagnosis had increased to 0.12 ± 0.05 mmol/l at one month and 0.13 ± 0.06 mmol/l at 3 months. These values were significantly lower ($p < 0.001$) than in the non-epidemic patients at the same time points: 0.17 ± 0.08 nmol/l; $p < 0.001$, 0.23 ± 0.11 nmol/l; $p < 0.001$, and 0.22 ± 0.10 nmol/l. Values of insulin antibodies (U/l) of 0.06 ± 0.03, 0.05 ± 0.05 and 0.17 ± 0.10 in the epidemic group compared to 0.014 ± 0.015, 0.02 ± 0.01, and 0.04 ± 0.04 in the non-epidemic group at the same aforementioned time points also showed significant differences ($p < 0.001$). Differences in these variables between the two groups continued until four years after diagnosis. Significant differences were also observed in the values of haemoglobin $A_{1c}$ and HLA-DR phenotype frequencies in the two groups. The results suggest that children with Type 1 diabetes can be divided into two sub-groups with different early clinical course which might depend on a different aetiology, related both to seasonal variation at diagnosis and to a genetic heterogeneity.

**Key words:** Type 1 (insulin-dependent) diabetes mellitus, seasonal variation, epidemic, non-epidemic, C-peptide, insulin antibodies, haemoglobin $A_{1c}$, HLA-DR types.

The aetiology of Type 1 (insulin-dependent) diabetes mellitus still remains an enigma – more so, since several reports [1–6] have provided substantial evidence that there may perhaps be more than one variety of the disease probably triggered by more than one mechanism in genetically susceptible individuals. This has made epidemiological studies even more necessary in our effort to define the aetiology of the disease. Because of this necessity, investigators in different countries [7–12] and in a number of continents [13–17] have performed epidemiological studies and presented their findings on the seasonality of the disease. But the reason for the seasonal trend, the exact share of it by each of the two sexes and the age groups in which seasonality does occur are yet to be elucidated.

A few elegant reports [2, 7, 18] have stated the opinion that there is a higher incidence in males than in females in certain age groups while the contrary is the case [8, 19] in a few other locations. B. Christau et al. [7] reported that seasonal variation was observed in subjects 0–29 years of age at diagnosis but hinted that no such findings could be demonstrated in sub-groups. A Dutch group [12] found seasonality in patients over 10 years of age at diagnosis while an earlier Swedish group [20] observed seasonality in all age groups studied except in patients younger than four years at diagnosis. R. M. Erhlich et al. [9] noted low and high monthly peaks in some groups of patients studied but cautioned that these may occur in certain years but not in others. However, in a study of 522 patients, R. West et al. [21] found seasonality to occur from year to year and a peak in November for patients under five years of age at diagnosis.

J. M. MacDonald et al. [22] believe that increased carbohydrate intolerance in the autumn and winter are the cause of the seasonality of the disease while other

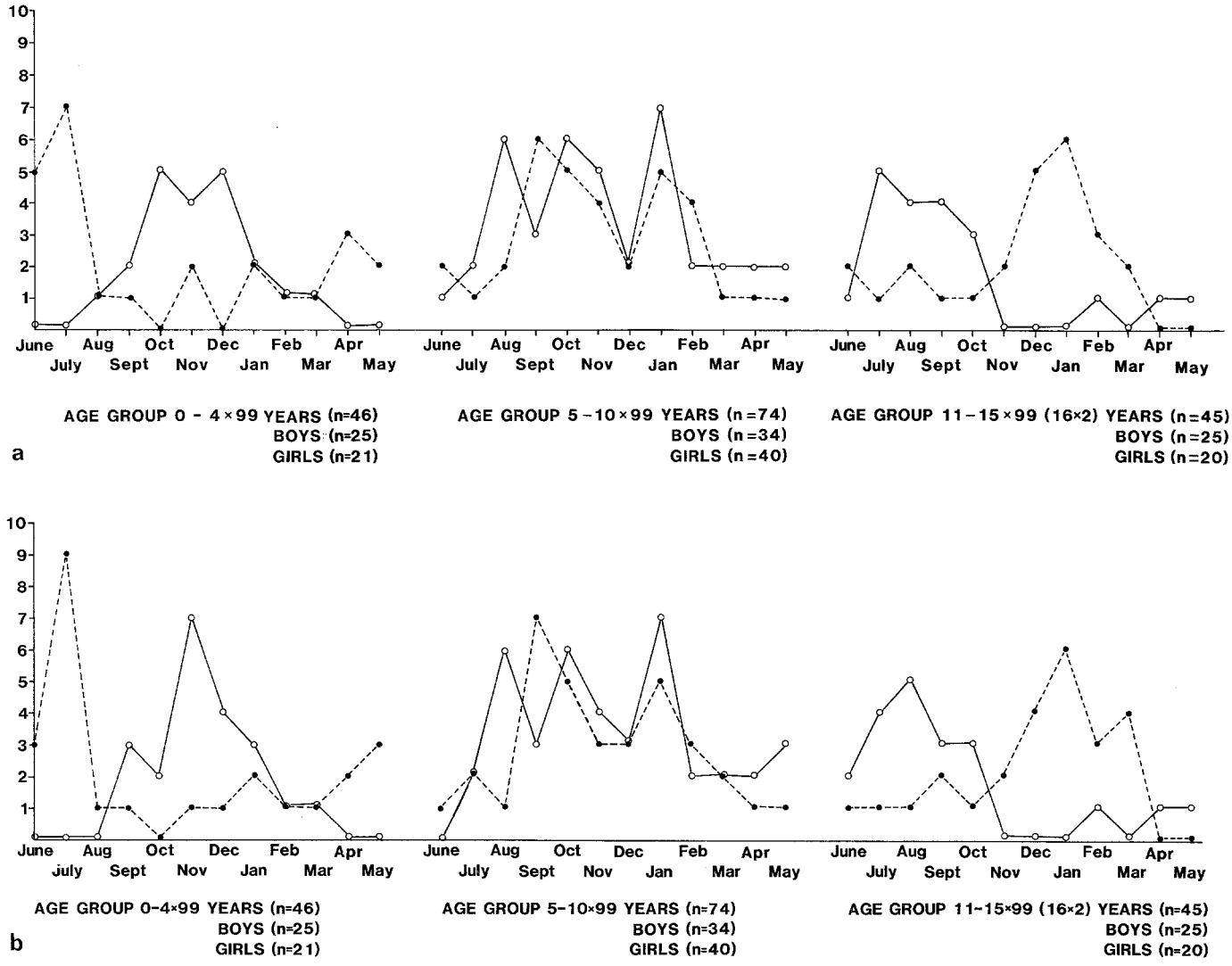


**Fig. 1.** **a** Seasonal variation in onset of symptoms in different age groups and different sexes. ○——— = girls; ●- - - = boys **b** Seasonal variation in diagnosis in different age groups and different sexes. ○——— = girls; ●- - - = boys

investigators [2, 4, 6, 16, 23] have laid emphasis on viral entities. It has as well been reported [5] that the seasonality of Type 1 diabetes mellitus is a question of the presence or absence of the HLA-DR antigen, which our own studies indicated earlier [24].

It is our belief, however, that in order to know more about the seasonality of Type 1 diabetes, a knowledge of what happens after the patient has become diabetic is valuable. Sometimes the preceeding mechanism of a disease process is better understood from the consequences that result. Any speculation on the possible cause of seasonality of Type 1 diabetes without a knowledge of what happens in the internal milieu of the two groups of patients (epidemic and non-epidemic groups) would be based on an incomplete premise. Accordingly, our present study compares (a) the duration of symptoms between onset and diagnosis, (b) the frequency of ketonuria at diagnosis in the two groups, (c) the values of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ in the two groups, and (d) the frequencies of HLA-DR 3/4 phenotypes in the two groups (epidemic and non-epidemic) of patients.

## Subject and methods

### *Patients*

All cases of Type 1 diabetes mellitus from the catchment area of the Department of Paediatrics, University Hospital, Linköping diagnosed between 1970 and 1986 (inclusive) and still being treated at the clinic were included in this 17-year retrospective study. One hundred sixty-five patients met our criteria on the basis of age at diagnosis, month and season of diagnosis, length of symptoms between onset and diagnosis and availability of complete clinical data and evolutionary history of disease. They range in age from 0.8-16.2 years (mean ± SD, 7.6 ± 4.1 years) at diagnosis as two boys were diagnosed over the age of 15.99 (16.0 and 16.2 years). There were 83 boys (age: range 0.8-16.2 years, mean ± SD: 7.7 − 4.5 years) and 82 girls (age: range 0.8-15.4 years; mean ± SD: 7.4 ± 3.8 years).

## Methods

There was a seasonal variation of diagnosis in all age groups (Fig. 1). All subjects were placed in one of two groups (epidemic and non-epidemic) in accordance with the month of diagnosis. But even then, there were still patients who had such a long duration of symptoms (greater than 3 weeks) before diagnosis that, even though they were diagnosed during peak months, they were really non-epidemic by our definition. For example, three patients (two boys aged 14.9 and 15.4 years and one girl aged 13.5 years) had symptoms (polydipsia, polyuria, unusual nocturia and weakness) suggestive of diabetes while on holidays, and it was not until they returned to Linköping that their disease was diagnosed. Therefore, these cases because of long duration of symptoms, although diagnosed during epidemic periods, were justifiably placed with the non-epidemic group (details illustrated in Fig. 2). On the basis of age at diagnosis, the patients were again divided into three groups, yielding 46 patients in the 0–4.99 year age group, 74 patients in the 5–10.99 year age group and 45 patients in the 11–16.99 year age bracket.

The patient files provided complete clinical data. Values of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ were obtained every 3 months. There serum C-peptide values were determined according to Heding [25] both fasting and in response to a standardised breakfast at regular intervals [26]. Insulin antibodies were determined according to Christiansen [27].

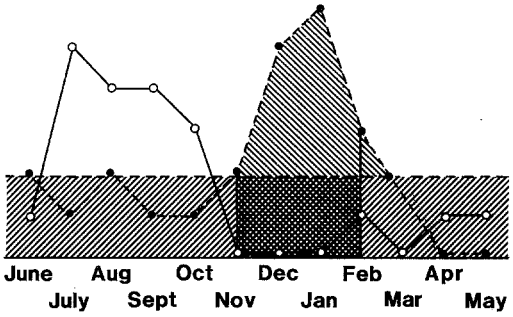


**Fig. 2.** A period with high incidence includes both the pure epidemic cases and a group of non-epidemic cases with onset evenly spread over the year. O----- = girls; ●- - - = boys; ▨ = non-epidemic cases; ▧ = pure epidemic cases; ▩ = non-epidemic cases which cannot be separated from the epidemic. Age group 11–16.2 years ($n=45$); boys ($n=25$); girls ($n=20$)

## Statistical analysis

Student's t-test and the $Chi^2$ technique were utilised to test the level of significance between variables with a $p<0.05$ and a $p<0.001$ as the lowest and highest level of significance respectively. We also analysed the data with multiple regression and used the Kolmogorov-Smirnow estimation procedure to test how co-varying variables are associated with seasonality.

# Results

## Seasonal variation

It is evident from Figure 1 that, regarding both onset of symptoms and diagnosis, seasonality occurred in all age groups in both sexes. In terms of onset of symptoms,

**Table 1.** Duration of symptoms before diagnosis and ketonuria: epidemic vs non-epidemic patients

| Group of patients | *n* | Duration of symptoms mean ± SD (days) | *p*-value | Ketonuria (1+ −3+ ++) | *p*-value |
|---|---|---|---|---|---|
| Epidemic patients | 69 | 13.2 ± 8.1 | | 88.40% (61/69) | |
| | | | $p<0.001$ | | $p<0.06$ |
| Non-epidemic patients | 96 | 23.0 ± 10.3 | | 71.88% (69/96) | (NS) |
| Patients 11–16.99 years of age | | | | | |
| Epidemic | 14 | 16.5 ± 9.7 | | 79% (11/14) | |
| | | | $p<0.001$ | | NS |
| Non-epidemic | 31 | 26.8 ± 11.4 | | 54.54% (17/31) | |
| Patients 5–10.99 years of age | | | | | |
| Epidemic | 39 | 13.5 ± 8.1 | | 89.74% (35/39) | |
| | | | $p<0.001$ | | NS |
| Non-epidemic | 35 | 25.2 ± 12.3 | | 74.29% (21/35) | |
| Patients 0–4.99 years of age | | | | | |
| Epidemic | 16 | 10.3 ± 6.6 | | 94% (15/16) | |
| | | | $p<0.001$ | | NS |
| Non-epidemic | 30 | 16.9 ± 7.3 | | 90% (27/30) | |

*n* = number of patients; NS = not significant

**Table 2.** Duration of symptoms before diagnosis and history of ketonuria in the Type 1 (insulin-dependent) diabetic patients studied ($n=165$)

| Group of patients | *n* | Duration of symptoms mean ± SD (days) | *p*-value | Ketonuria (1+ −3+ ++) | *p*-value |
|---|---|---|---|---|---|
| All patient studied | 165 | 17.6 ± 12.3 | NS | 80% (131/16) | |
| Boys in the group | 83 | 16.6 ± 8.5 | NS | 72% (60/83) | |
| Girls in the group | 82 | 18.8 ± 10.0 | $p<0.07$ | 88% (71/82) | |
| Children 11–16.99 years | 45 | 20.1 ± 15.0 | NS | 62% (28/45) | |
| Children 5–10.99 years | 74 | 17.6 ± 6.7 | $p<0.11$ | 82% (61/74) | $p<0.001$ |
| Children 0–4.99 years | 46 | 13.8 ± 4.2 | $p<0.001$ | 91% (42/46) | |

*n* = number of patients; NS = not significant

**Table 3.** Mean ± SD of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ in the indicated group of patients

| Duration | C-peptide (mean ± SD) | | *p-value* | Insulin antibodies (mean ± SD) | | *p-value* | $HbA_{1c}$ (mean ± SD) | | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| | Epidemic patients | Non-epidemic patients | | Epidemic patients | Non-epidemic patients | | Epidemic patients | Non-epidemic patients | |
| Diagnosis | 0.11 ± 0.05 | 0.17 ± 0.08 | $p<0.001$ | 0.06 ± 0.03 | 0.014 ± 0.015 | $p<0.001$ | 14.42 ± 2.06 | 11.30 ± 1.39 | $p<0.001$ |
| 1 month | 0.12 ± 0.05 | 0.23 ± 0.11 | $p<0.001$ | 0.05 ± 0.05 | 0.02 ± 0.01 | $p<0.001$ | 8.84 ± 0.66 | 8.35 ± 0.78 | $p<0.001$ |
| 3 months | 0.13 ± 0.06 | 0.22 ± 0.10 | $p<0.001$ | 0.17 ± 0.10 | 0.04 ± 0.04 | $p<0.001$ | 8.78 ± 0.55 | 8.09 ± 0.46 | $p<0.001$ |
| 9 months | 0.10 ± 0.07 | 0.15 ± 0.07 | $p<0.001$ | 0.26 ± 0.14 | 0.12 ± 0.11 | $p<0.001$ | 9.13 ± 0.82 | 8.57 ± 0.65 | $p<0.001$ |
| 18 months | 0.09 ± 0.04 | 0.12 ± 0.07 | $p<0.001$ | 0.36 ± 0.25 | 0.18 ± 0.11 | $p<0.001$ | 9.62 ± 2.04 | 9.03 ± 1.61 | $p<0.0.04$ |
| 3 years | 0.07 ± 0.04 | 0.08 ± 0.04 | $p<0.12$ NS | 0.41 ± 0.23 | 0.25 ± 0.18 | $p<0.001$ | 9.66 ± 2.08 | 9.41 ± 1.87 | $p<0.42$ NS |
| 4 years | 0.05 ± 0.03 | 0.06 ± 0.05 | $p<0.14$ NS | 0.45 ± 0.35 | 0.34 ± 0.21 | $p<0.006$ | 10.34 ± 2.34 | 10.05 ± 1.59 | $p<0.34$ NS |

All patients studied 0–16.2 years at diagnosis ($n=165$); epidemic patients ($n=69$); non-epidemic patients ($n=96$).
NS = not significant

**Table 4.** Mean ± SD of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ in the indicated groups of patients

| Duration | C-peptide (mean ± SD) | | *p-value* | *Insulin antibodies (mean* ± SD) | | *p-value* | $HbA_{1c}$ (mean ± SD) | | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| | Epidemic patients $n=14$ | Non-epidemic patients $n=31$ | | Epidemic patients $n=14$ | Non-epidemic patients $n=31$ | | Epidemic patients $n=14$ | Non-epidemic patients $n=31$ | |
| Diagnosis | 0.13 ± 0.08 | 0.20 ± 0.06 | $p<0.001$ | 0.05 ± 0.03 | 0.003 ± 0.005 | $p<0.001$ | 14.74 ± 2.44 | 11.04 ± 1.22 | $p<0.001$ |
| 1 month | 0.14 ± 0.06 | 0.27 ± 0.12 | $p<0.001$ | 0.05 ± 0.06 | 0.02 ± 0.007 | $p<0.001$ | 9.07 ± 0.90 | 8.32 ± 0.73 | $p<0.001$ |
| 3 months | 0.19 ± 0.10 | 0.29 ± 0.14 | $p<0.001$ | 0.08 ± 0.05 | 0.04 ± 0.03 | $p<0.001$ | 8.52 ± 0.27 | 7.52 ± 0.51 | $p<0.001$ |
| 9 months | 0.18 ± 0.08 | 0.21 ± 0.10 | $p<0.33$ NS | 0.08 ± 0.06 | 0.05 ± 0.04 | $p<0.04$ | 8.62 ± 0.61 | 8.46 ± 0.47 | $p<0.03$ NS |
| 18 months | 0.15 ± 0.09 | 0.16 ± 0.14 | NS | 0.16 ± 0.26 | 0.13 ± 0.08 | NS | 9.44 ± 2.59 | 9.06 ± 2.47 | NS |
| 3 years | 0.12 ± 0.08 | 0.13 ± 0.11 | NS | 0.19 ± 0.13 | 0.17 ± 0.12 | NS | 9.51 ± 2.50 | 9.28 ± 2.49 | NS |
| 4 years | 0.10 ± 0.05 | 0.09 ± 0.08 | NS | 0.21 ± 0.12 | 0.23 ± 0.20 | NS | 10.04 ± 3.60 | 10.41 ± 2.70 | NS |

Children 11–16.2 years of age at diagnosis ($n=45$).
$n$ = number of patients; NS = not significant

**Table 5.** Mean ± SD of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ in the indicated groups of patients

| Duration | C-peptide (mean ± SD) | | *p-value* | Insulin antibodies (mean ± SD) | | *p-value* | $HbA_{1c}$ (mean ± SD) | | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| | Epidemic patients $n=39$ | Non-epidemic patients $n=35$ | | *Epidemic* patients $n=39$ | *Non-epidemic* patients $n=35$ | | Epidemic patients $n=39$ | Non-epidemic patients $n=35$ | |
| Diagnosis | 0.11 ± 0.04 | 0.18 ± 0.13 | $p<0.001$ | 0.06 ± 0.04 | 0.02 ± 0.02 | $p<0.001$ | 14.12 ± 1.58 | 11.32 ± 1.45 | $p<0.001$ |
| 1 month | 0.13 ± 0.06 | 0.5 ± 0.15 | $<0.001$ | 0.03 ± 0.03 | 0.01 ± 0.04 | $p<0.001$ | 8.80 ± 0.43 | 8.3 ± 0.81 | $p<0.001$ |
| 3 months | 0.11 ± 0.05 | 0.21 ± 0.09 | $p<0.001$ | 0.08 ± 0.05 | 0.04 ± 0.05 | $p<0.001$ | 9.25 ± 1.10 | 8.36 ± 0.56 | $p<0.001$ |
| 9 months | 0.09 ± 0.07 | 0.17 ± 0.10 | $p<0.001$ | 0.13 ± 0.10 | 0.07 ± 0.10 | $p<0.01$ | 9.39 ± 1.49 | 8.46 ± 1.02 | $p<0.001$ |
| 18 months | 0.08 ± 0.03 | 0.12 ± 0.06 | $p<0.01$ | 0.28 ± 0.12 | 0.13 ± 0.04 | $p<0.001$ | 9.55 ± 1.26 | 8.68 ± 1.22 | $p<0.01$ |
| 3 years | 0.04 ± 0.03 | 0.07 ± 0.04 | $p<0.05$ | 0.44 ± 0.26 | 0.24 ± 0.15 | $p<0.01$ | 9.65 ± 2.27 | 9.05 ± 2.06 | $p<0.05$ |
| 4 years | 0.03 ± 0.02 | 0.05 ± 0.06 | $p<0.05$ | 0.46 ± 0.14 | 0.29 ± 0.11 | $p<0.05$ | 10.28 ± 2.14 | 9.65 ± 1.13 | NS |

Children 5–10.99 years of age at diagnosis ($n=74$).
$n$ = number of patients; NS = not significant

**Table 6.** Mean ± SD of C-peptide, insulin antibodies and haemoglobin $A_{1c}$ in the indicated groups of patients

| Duration | C-peptide (mean ± SD) | | *p-value* | Insulin antibodies (mean ± SD) | | *p-value* | $HbA_{1c}$ (mean ± SD) | | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| | Epidemic patients $n=16$ | Non-epidemic patients $n=30$ | | Epidemic patients $n=16$ | Non-epidemic patients $n=30$ | | Epidemic patients $n=16$ | Non-epidemic patients $n=30$ | |
| Diagnosis | 0.08 ± 0.04 | 0.14 ± 0.06 | $p<0.001$ | 0.06 ± 0.03 | 0.02 ± 0.02 | $p<0.001$ | 14.41 ± 2.15 | 11.55 ± 1.51 | $p<0.001$ |
| 1 month | 0.09 ± 0.04 | 0.15 ± 0.08 | $p<0.01$ | 0.08 ± 0.06 | 0.03 ± 0.02 | $p<0.001$ | 8.66 ± 0.64 | 8.44 ± 0.80 | $p<0.32$ NS |
| 3 months | 0.08 ± 0.04 | 0.17 ± 0.07 | $p<0.001$ | 0.34 ± 0.21 | 0.05 ± 0.03 | $p<0.001$ | 9.16 ± 0.28 | 8.16 ± 0.28 | $p<0.001$ |
| 9 months | 0.04 ± 0.02 | 0.08 ± 0.05 | $p<0.01$ | 0.56 ± 0.25 | 0.25 ± 0.20 | $p<0.001$ | 9.38 ± 0.35 | 8.76 ± 0.52 | $p<0.01$ |
| 18 months | 0.04 ± 0.03 | 0.04 ± 0.02 | NS | 0.62 ± 0.39 | 0.27 ± 0.21 | $p<0.001$ | 9.88 ± 2.25 | 9.36 ± 1.15 | $p<0.50$ NS |
| 3 years | 0.04 ± 0.02 | 0.05 ± 0.03 | NS | 0.60 ± 0.31 | 0.36 ± 0.26 | $p<0.01$ | 9.78 ± 1.42 | 9.90 ± 1.10 | NS |
| 4 years | 0.03 ± 0.02 | 0.04 ± 0.03 | NS | 0.78 ± 0.15 | 0.43 ± 0.17 | $p<0.001$ | 10.70 ± 1.29 | 10.09 ± 0.87 | NS |

Children 0–4.99 years of age at diagnosis ($n=46$).
$n$ = number of patients; NS = not significant

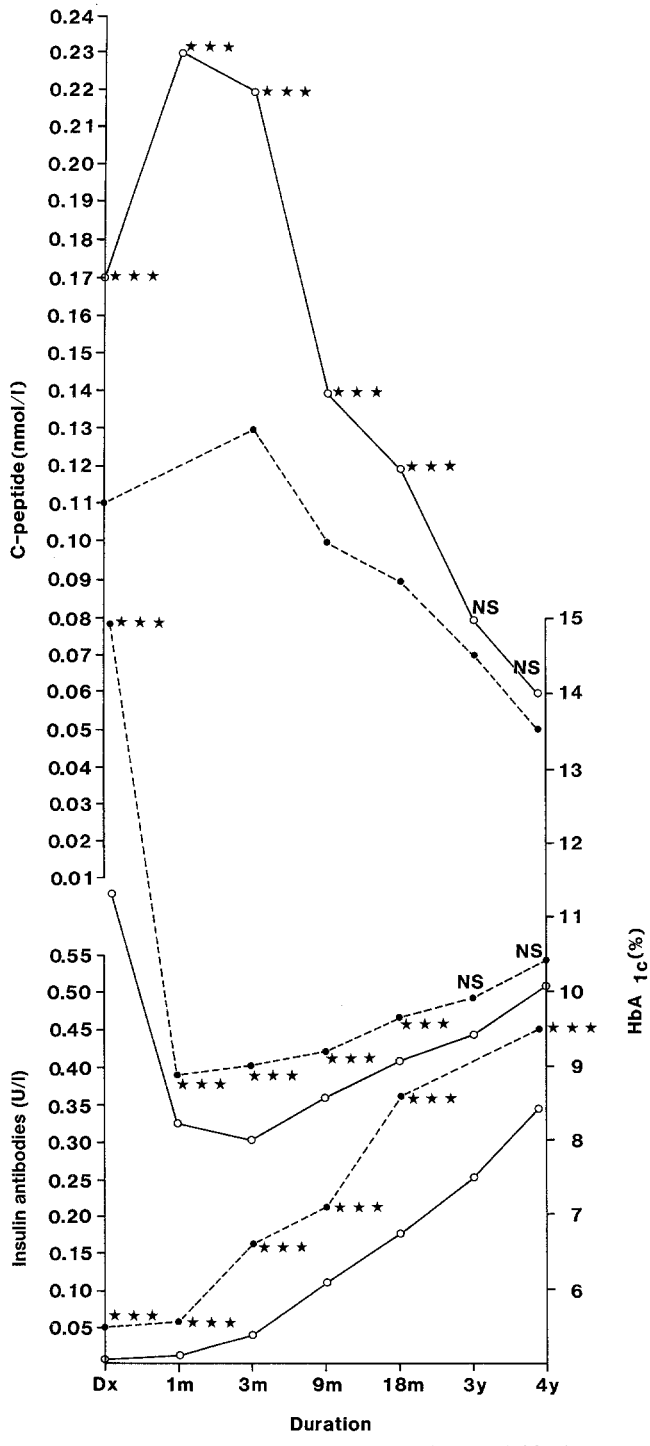


**Fig. 3.** C-peptide, insulin antibodies and haemoglobin $A_{1c}$: mean values compared between the epidemic ($n=69$) and non-epidemic ($n=96$) patients. ***$=p<0.001$; **$=p<0.01$; *$=p<0.05$; NS = non-significant; ○——— = non-epidemic patients; ●- - - = epidemic patients; Dx = diagnosis; m = months; y = years

one peak (June through July) was seen in boys and another peak (October through December) was observed in girls in the 0–4.99 year age bracket. In terms of diagnosis, the peak observed in boys was even more narrow than the peak for onset of symptoms. In November through December, a peak was seen in girls.

Boys and girls in the 5–10.99 year age group showed peaks in August through January both in onset of symptoms and diagnosis. However, in the 11–16.2 year age group, an unequal peak (December, January and March) was seen in boys, but girls had a peak in July through August with regards to onset of symptoms. In terms of diagnosis, the boys in this age group had a prominent peak in January, while girls had the same peak as in onset of symptoms. In effect, seasonality of both onset of symptoms and diagnosis was observed in all age groups in both sexes in our study.

When the duration of symptoms before a diagnosis is considered (Tables 1, 2), the epidemic group had a shorter ($13.2 \pm 8.1$ days) duration of symptoms as compared to $23.0 \pm 10.3$ days ($p<0.001$) in the non-epidemic patients. Patients who were younger (0–4.99 years) in age at diagnosis had shorter a ($13.8 \pm 4.2$ days) duration of symptoms than patients who were older (11–16.2 years) at diagnosis ($20.1 \pm 15.0$ days, $p<0.001$). Therefore, in our study, duration of symptoms before diagnosis relates to age of patients in a negative manner.

### *Ketonuria*

Ketonuria (1+ −3+ + +) at diagnosis was found in 79% (131/165) of all the patients. In the epidemic group, 88.4% (61/69) had ketonuria as compared to 71.9% (69/96, $p<0.06$) in the non-epidemic patients. The youngest group of patients (0–4.99 years at diagnosis) had more cases with ketonuria (91%, 41/46) than the oldest group (11–16.2 years) of patients with only 62% (28/45, $p<0.001$) ketotic cases (details shown in Tables 1 and 2).

### *C-peptide, insulin antibodies and $HbA_{1c}$*

It can be seen from the Figures and Tables 3–6 that the differences in C-peptide, insulin antibodies and haemoglobin $A_{1c}$ between the epidemic and the non-epidemic patients in the first four years of disease are overwhelming. Thus, in all age groups the non-epidemic patients had higher C-peptide, lower $HbA_{1c}$, and less insulin antibodies than the epidemic patients. After the fourth year, there was no difference in the values of the three variables in the two groups.

### *HLA-DR 3/4 phenotypes*

Results of HLA-DR phenotypes available in 78.8% (130/165) of our patients show that 90.8% (118/130) of the patients are DR-3 and 4 heterozygous. In the epidemic patients, HLA-DR 4 phenotype was more frequent (88%; 44/50) than in the non-epidemic (63.8%; 44/80), $p<0.001$) group of patients. Details are shown in Table 7.

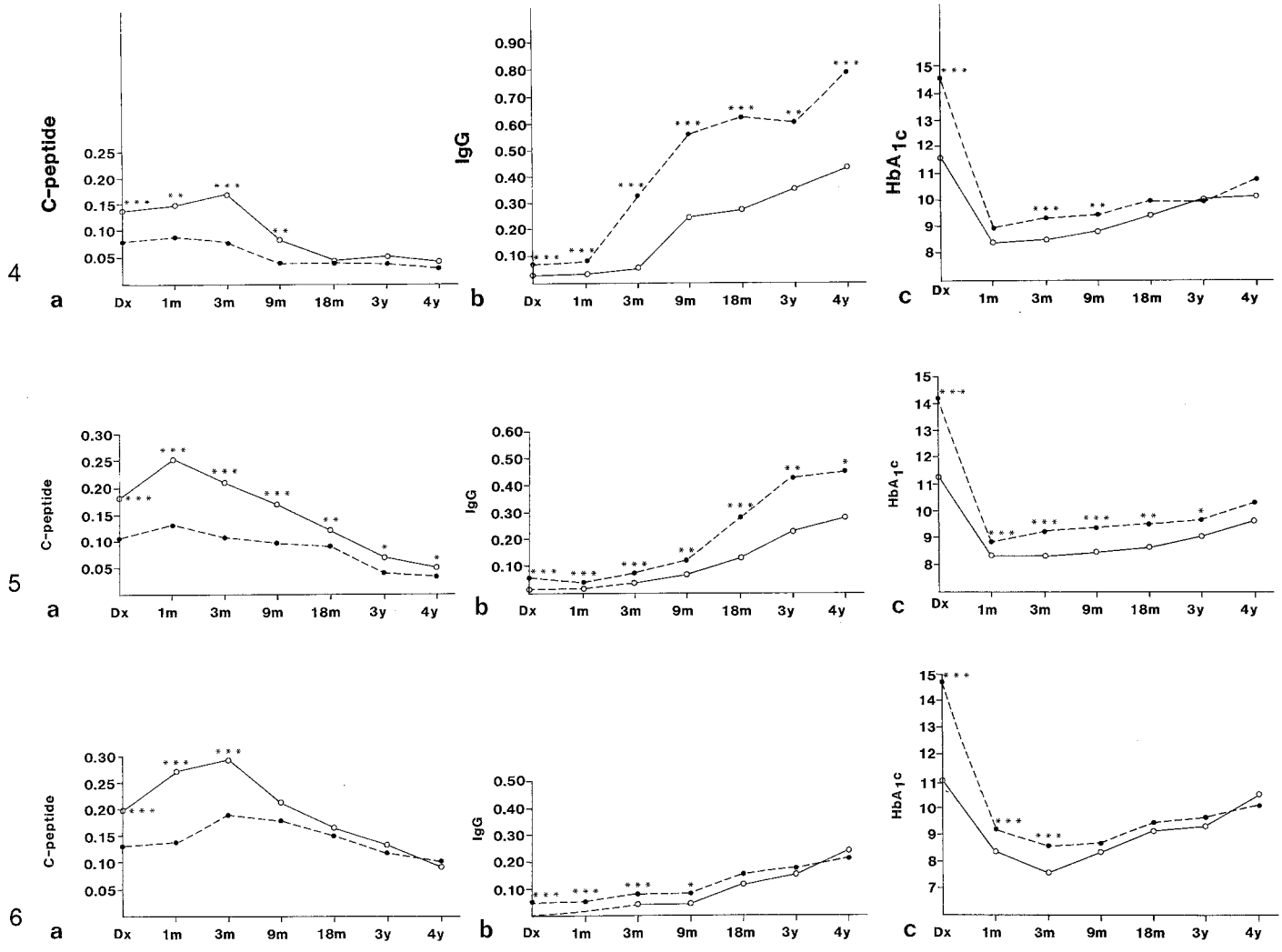


**Fig. 4a-c.** Mean values of C-peptide, insulin antibodies (IgG) and $HbA_{1c}$ in patients 0-4.99 years of age at diagnosis ($n=46$). **a** C-peptide. **b** Insulin antibodies. **c** $HbA_{1c}$. O——— = non-epidemic patients; ●- - - = epidemic patients; $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

**Fig. 5a-c.** Mean values of C-peptide, insulin antibodies (IgG) and $HbA_{1c}$ in patients 5-10.99 years of age at diagnosis ($n=74$). **a** C-peptide. **b** Insulin antibodies. **c** $HbA_{1c}$. O——— = non-epidemic patients; ●- - - = epidemic patients; $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

**Fig. 6a-c** Mean values of C-peptide, insulin antibodies (IgG) and $HbA_{1c}$ in patients 11-16.2 years of age at diagnosis ($n=45$). **a** C-peptide. **b** Insulin antibodies. **c** $HbA_{1c}$. O——— = non-epidemic patients; ●- - - = epidemic patients; $*=p<0.05$; $**=p<0.01$; $***=p<0.001$

## Discussion

According to our Swedish medical system, all children with diabetes living within the catchment area of our hospital are diagnosed and treated here. We, therefore, have a group of patients which is representative of a defined population which, in turn, is representative of the Swedish population, thus reducing the problem of referral bias to a minimum. Furthermore, as there were no missing data regarding symptoms at onset, we feel able to draw some conclusions based on our retrospective data.

We observed seasonal variation in onset of symptoms and diagnosis in all patient groups studied in contrast to one previous report [2]. Our observation that autumn, winter and early spring are the seasons of highest incidence of Type 1 diabetes differs little from that observed by others [7, 10, 14]. However, we have found that boys and girls have their incidence peaks during different months in different age groups. Thus, boys diagnosed before the age of 5 actually had a peak in the months of June through July; while a peak was observed in October through December in girls in that age group.

Our study shows that seasonal variation in onset of symptoms and diagnosis of Type 1 diabetes is real. We are convinced by our results that a previous opinion [7] - that lack of prompt medical attention and diagnosis during the summer months can account for the seaso-

**Table 7.** HLA-DR3 and DR4 phenotype frequencies in patients 0–16.2 years at diagnosis ($n=130$)

| Groups of patients | Number typed | % with DR3 | *p*-value | % with DR4 | *p*-value | % with DR3 and/or 4 |
|---|---|---|---|---|---|---|
| All patient typed | 130 | 52.31% (68/130) | | 73.08% (95/130) | | 90.77% (118/130) |
| Boys 0–16.99 years | 68 | 44.12% (30/68) | $p<0.05$ | 80.88% (55/68) | NS | 92.65% (63/68) |
| Girls 0–16.99 years | 62 | 61.29% (38/62) | | 64.52% (40/62) | | 88.71% (55/62) |
| Patients 0–16.99 years | | | | | | |
| Epidemic patients | 50 | 48% (24/50) | | 88% (44/50) | $p<0.001$ | 94% (47/50) |
| Non-epidemic patients | 80 | 55% (44/80) | NS | 63.75% (51/80) | | 88.75% (71/80) |
| Patients 0–4.99 years | | | | | | |
| Epidemic patient | 13 | 46.15% (6/13) | | 100% (13/13) | | 100% (13/13) |
| Non-epidemic patients | 21 | 52.38% (11/21) | NS | 61.91% (13/21) | $p<0.02$ | 90.48% (19/21) |
| Patients 5–10.99 years | | | | | | |
| Epidemic patients | 24 | 45.83% (11/24) | | 83.33% (20/24) | | 91.67% (22/24) |
| Non-epidemic patients | 31 | 54.84% (17/31) | NS | 61.29 (19/31) | NS | 87.10% (27/31) |
| Patients 11–16.99 years | | | | | | |
| Epidemic patients | 13 | 53.85% (7/13) | | 84.62% (11/13) | | 92.31% (12/13) |
| Non-epidemic patients | 28 | 57.14% (16/28) | NS | 67.86% (19/28) | NS | 89.29% (25/25) |

NS = non significant

nality of diagnosis of Type 1 diabetes – should be rejected. The fact that seasonality of Type 1 diabetes occurs both in onset of symptoms as well as in diagnosis justifies the rejection of such a simplistic explanation.

The duration of symptoms between onset and diagnosis related to the age of patients in a negative manner which confirms earlier reports [2, 28]. In addition, however, we have shown that those patients diagnosed during peak months have shorter duration of symptoms than those with their onset during low incidence periods of the year. This indicates that the epidemic and non-epidemic patients differ with regard to the destructive process leading to the manifestation of the disease. This is further supported by the results regarding C-peptide and the related finding that the epidemic patients almost always have ketonuria at diagnosis. The lower values of $HbA_{1c}$ in the non-epidemic patients is probably a secondary phenomenon of their better residual insulin secretion.

The significantly low levels of C-peptide at diagnosis and up to four years later in the epidemic patients correspond well with the expected higher values of insulin antibodies. This confirms earlier reports [1, 29, 30] that in Type 1 diabetes patients, values of C-peptide relate negatively to insulin antibodies, which might be due to a negative effect of the insulin antibodies. It is impossible to know whether the insulin antibodies have a negative effect on the B cells, or whether they simply reflect an ongoing, more active immunological process.

HLA-DR 3/4 phenotypes seem to be associated with accelerated B-cell destruction and epidemicity of Type 1 diabetes as shown in Table 7. Several previous reports [1, 5, 24, 31] have indicated that the genetic susceptibility of Type 1 diabetes is associated to the HLA-D locus. Our present data further support the proposed hypothesis that there is a genetic heterogeneity. The increased frequency of HLA-DR 4 in the epidemic patients would conform well with the idea that DR4 increases the susceptibility for virus infections while DR3 would be more associated with a slowly progressive autoimmune type of diabetes reasonably represented throughout the year among the non-epidemic cases [24].

From the observable differences in the two groups (epidemic and non-epidemic) reported here, one is tempted to conclude that: (a) Type 1 diabetes mellitus evolves or at least is made manifest as an acute problem in the epidemic but not as much in the non-epidemic patients; (b) the seemingly acute lesion (implied by the rapidly worsening condition at diagnosis) and the clustering of cases (evident from persistent epidemicity) of Type 1 diabetes, portrays the characteristics of a disease triggered or elicited by an infective mechanism (most probably viruses) in some of the genetically susceptible individuals; (c) the epidemic form of Type 1 diabetes may be a situation where viruses, the immune system and genetics play unequal roles in producing the disease; (d) there is a more accelerated B-cell loss in the epidemic patients; and (e) there may be at least two aetiological variants of Type 1 diabetes, the mechanism in genetically susceptible individuals of which may differ.

Our study shows that the differences between the epidemic and non-epidemic groups of Type 1 diabetes patients at diagnosis and up to four years later are pronounced. One of the authors, supported by others [2–6] had reported earlier that there may be more than one aetiological variant of Type 1 diabetes elicited in genetically susceptible individuals; probably by more than one mechanism. Our present study confirms that observation.

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