Type 1 diabetes-related epidemiological, clinical and laboratory findings

An evaluation with special regard to autoimmunity in children

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ABSTRACT

Objectives: To evaluate our data related with epidemiologic features, clinical presentation, and laboratory findings in children with type 1 diabetes mellitus (DM1) and to compare specific characteristics of immune-mediated subtype (DM 1A) with idiopathic one (DM 1B).

Methods: We classified 115 children with DM1 according to the presence (DM 1A, n=77) or absence (DM 1B, n=38) of diabetes-related autoantibodies in Akdeniz University Hospital, Turkey from January 2000 to December 2005.

Results: A total of 43 patients (37%) in the whole group, had onset of DM1 during the winter months and the lowest frequency occurred in summer (p<0.005). The duration of breast-feeding, introduction time of cow's milk, and seasonal distribution of birth-month or onset of disease did not significantly differ in both groups. When compared with patients who had no documented honeymoon period, the patients who had a documented honeymoon period had lower HbA1c levels (p<0.01) at the onset. A large percentage of patients with DM 1A were positive for glutamic acid decarboxylase antibody (GAD65).

Conclusion: There was no significant difference between patients with DM 1A and DM 1B with respect to epidemiologic features, and clinical presentation suggested that these factors do not play a major role either in creating a disease-initiating effect or in the development of islet autoimmunity. However, determination of GAD65 with HbA1c levels at the onset of the disease may ensure some useful information regarding clinical course.

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Type 1 diabetes mellitus (DM1), is characterized by an autoimmune process that results from pancreatic beta-cells destruction in subjects with genetic susceptibility to the disease and exposure to still undefined environmental factors. Epidemiological studies from all parts of the world have reported increasing incidence of DM1.[1,2] The concordance of DM1 among monozygotic twins is 21-70%, suggesting that environmental factors play an important role in the etiology.[3-7] An environmental factor could be involved either by initiating or suppressing autoimmunity, or by altering its progression once autoimmunity is established.[3-5,8,9] Some authors have reported variations in DM1 incidence related with early infant diet, duration of breast feeding, month of birth, vitamin supplementation, and pre- and postnatal infections.[3,4,10,11] However, the epidemiological evidences are still inconsistent, and the environmental factors may differ substantially from population to population. Moreover, several studies suggest the relationship between some clinical characteristics and course of DM1; others do not support this relationship.[12-14] In this study, we aimed to evaluate our data on epidemiologic features, clinical presentation, and some specific laboratory findings in children with DM1, and to compare these characteristics of immune-mediated subtype (DM 1A) with that of the idiopathic one (DM 1B). We also aimed to establish the relationship between these findings and clinical course of DM1.

Methods. A retrospective study was undertaken in the Akdeniz University Hospital in Antalya, Southwest region of Turkey. In this study, we recruited 115 children (66 girls and 49 boys) who were followed in our Pediatric Endocrinology Department. These children had developed diabetes at <17 years of age between January 2000 and

December 2005. At the time of onset of overt diabetes, all patients were hospitalized. From their hospital records, we reviewed the following information; 1. Epidemiologic features; age, gender, breast-feeding, introduction time of cow's milk, family history for consanguinity as well as for the presence of DM1 or type 2 diabetes mellitus (DM2), seasonality of onset of DM1 and birth-month, date of diagnosis. 2. Clinical features; anthropometric values (height standard deviation score [Hsds]; body mass index; [BMI]), information regarding infections within the last 3 months before the diagnosis (without distinction between viral or bacterial infections), presence of ketoacidosis, and honeymoon period. 3. laboratory findings; serum bicarbonate (HCO_3) and pH values, serum glucose level, glycosylated hemoglobin (HbA1c), thyroid-related antibodies (antibodies against to thyroglobulin [antiTg] and thyroid peroxidase [TPOAbs]), and diabetes-related autoantibodies including to islet cell (ICAs), to glutamic acid decarboxylase (GAD65), and to insulin (IAAs). All the 115 patients met the criteria of American Diabetes Association for DM1.[15] Informed written consent was obtained from the participating patients' families. Diabetic ketoacidosis (DKA) was defined as hyperglycemia above 200 mg/dl (≅11 mmol/L) and pH <7.30, or HCO_3 ≤15 mmol/L in the presence of ketonuria.[5] Patients who were suspected of DM2 or having maturity-onset diabetes of young, or Wolfram syndrome, or secondary DM, and the patients who had incomplete data were excluded from the study. The duration of overall breast-feeding was defined as the period when the child received maternal breast milk, regardless of other food supplements. The honeymoon period (clinical remission) was defined as an insulin requirement of <0.5 U/kg/day.[16] Hsds and BMI (kg/m^2) were also calculated. Within the first day of diagnosis, ICAs, GAD65, and IAAs were studied. The patients were divided into 2 groups according to presence of diabetes-related autoantibodies; 77 (67%) were DM 1A (Group 1) and 38 (33%) were DM 1B (Group 2). DM 1A was defined as having at least one diabetes-related autoantibody. The HbA1c levels were determined by using the turbidimetric inhibition immunoassay (TINIA) for the hemolyzed whole blood (Roche, Tina-quant, with a nondiabetic range 4.8-6.0%). GAD65 were measured by radioimmuno assay (Rad-med, CIS, GAD-AB kit), ICAs were measured by immunofluorescent assay (Binding Site-UK, substrate: monkey-pancreas), and IAAs were measured by radioimmuno assay (BioSource AIA kit, Belgium). Cut-off values were 1 U/MI for GAD65 and 7% for IAAs. ICAs test results were expressed as positive or negative. Results were evaluated as score 0 for negativity and score 1 for positivity. Anti-Tg and TPOAbs were measured by immunoassay (Roche Diagnostics, Elecsys Reagent Kit). Statistical analyses were performed using Statistical Package for Social Sciences (SPSS Version 13.0) software. Chi-square, t-test, Mann Whitney U, and Wilcoxon tests were used to compare the variables. Spearman and Pearson's correlation coefficient was used for assessment of association between 2 continuous variables. *P*-value of less than 0.05 was considered significant.

Results. The mean duration of DM1 in the patients was 1.7±1.5 years. The distribution of age at onset of the disease was shown in **Table 1.** Mean age at onset was 8.5 years (range; 1.3-16.0 years). The majority of the patients were older than 5 years old (83%) at diagnosis. The most common age period for the onset was 5.0-9.9 years in Group 1 and ≥10 years in Group 2 (p>0.05). The ratio of female to male was 1.4 for Group 1 and 1.2 for Group 2, with an insignificant difference (p>0.05, **Table 2**). When females were compared with males, there was no significant difference in terms of age at onset of the disease, seasonality of the birth-month and onset of the disease, infantile feeding type, duration of symptoms, values of BMI, serum glucose concentration, and HbA1c levels. There was no significant difference with respect to epidemiological, clinical, and laboratory findings at diagnosis between male patients with DM 1A and female ones with their respective patients with DM 1B (data not given). In the whole group, 21% of the patients had been exposed to cow's milk before age of 3 months, and the mean age at which cow's milk was introduced was 6.6±4.9 months. As shown in **Table 3**, total duration of breast-feeding and introduction time of cow's milk did not significantly differ in both groups. Only 2 patients were not been breast-fed. No significant correlation was found between the early introduction (≤3 months) to cow's milk and the duration of breast feeding on the age of onset of DM1, neither in DM 1A patients nor in DM 1B ones. Of the 115 children, 11.3% (n=13) had immediate consanguineous (first and second degree) parents, while the majority (88.7%) had no consanguineous parents. Family history of DM1 and DM2 were higher in Group 2 than that of Group 1, but only insignificantly (p>0.05). When the patients were divided into 2 groups according to presence (n=15) and absence (n=100) of family history of DM1, age at onset of the disease was 9.7±3.5 versus 8.5±3.6 years (p>0.05), and 9.3±3.5 versus 8.1±3.5 years (p>0.05) in the presence (n=41) and absence (n=74) of family history of DM2. Presence of paternal family history of DM1 or DM2 was 27% and 37% in Group 1 and 2 (p>0.05). Five (5%) of the 115 patients with DM1 had a diabetic father; 2 (2%) had one sibling with DM1. Among the 5 patients who had fathers with

Table 1 - Age distribution of patients in both groups.*

Age at onset	Group 1 n (%)	Group 2 n (%)
<5 years	13 (17)	6 (16)
5.0 - 9.9 years	37 (48)	15 (39)
≥10 years	27 (35)	17 (45)
Total	**77 (100)**	**38 (100)**

*p>0.05 for all comparisons

Table 2 - Brief epidemiological and clinical characteristics of patients in Group 1 and 2.*

Parameters	Group 1 (n=77) n (%)	Group 2 (n=38) n (%)
Gender		
Female	45 (58)	21 (55)
Male	32 (42)	17 (45)
Consanguinity	8 (10)	5 (13)
Season of birth		
Spring	21 (27)	7 (18)
Summer	19 (25)	11 (29)
Autumn	12 (16)	9 (24)
Winter	25 (32)	11 (29)
Season at diagnosis		
Spring	17 (22)	8 (21)
Summer	11 (14)	5 (13)
Autumn	26 (34)	5 (13)
Winter	23 (30)	20 (53)
Family history of type 1 diabetes mellitus	8 (10)	7 (18)
Family history of type 2 diabetes mellitus	32 (42)	19 (50)
Cesarean section	10 (13)	6 (16)
Duration of breast feeding <3 months	11 (14)	9 (24)
Cow's milk before 3 months	16 (21)	8 (21)
Presence of prodromal infections	34 (44)	18 (47)
Diabetic ketoacidosis at diagnosis	25 (33)	17 (45)
Documented honeymoon period	31 (40)	11 (29)
Hashimoto thyroiditis	9 (12)	1 (3)

*p>0.05 for all comparisons in Group 1 versus Group 2

Table 3 - Brief epidemiological, clinical and laboratory findings.

Parameters	Group 1 (n=77)	Group 2 (n=38)
Age (years)	10.5 ± 3.7	10.7 ± 4.1
Age at diagnosis (years)	8.7 ± 3.5	9.0 ± 3.8
Breast-feeding time (months)	12.3 ± 8.6	11.5 ± 8.5
Introduction time of cow's milk (months)	6.6 ± 5.3	6.5 ± 4.3
BMI at diagnosis (kg/m^2)	16.0 ± 3.1	16.2 ± 2.6
Onset of honeymoon period (months)	3.4 ± 2.6	7.1 ± 5.1
pH	7.31 ± 0.14	7.26 ± 0.16
Bicarbonate (mEq/L)	16.6 ± 7.6	13.1 ± 7.8
Serum glucose (mg/dL)	410.5 ± 137	385.8 ± 131
HbA1c (%)	12.7 ± 3.3	12.5 ± 3.4

Data are expressed as mean ± SD. Group 1 versus group 2 for all comparison were considered insignificant (p>0.05).

DM1, 3 of them were in Group 1. No patients had a diabetic mother or both parents being affected by the disease. Seasonal distribution of birth month was not found significantly different among the groups **(Table 2).** While autumn (September-November) and winter (December-February) were the most detected seasons at diagnosis in Group 1 patients, winter was the predominant season in Group 2, however, there was no significant relation between the seasonality at diagnosis and autoimmunity (p>0.05). In the whole group, 43 patients (37%) had onset of DM1 during the winter months and the lowest frequency occurred in summer (June-August, p=0.003). Mean Hsds was in normal range for both groups. BMIs were not found to be significantly different between boys and girls (16.0±2.4 kg/m^2 versus 16.2±3.2 kg/m^2, p>0.05). BMIs also showed no significant difference among the groups **(Table 3).** Fifty-two (45%) of the patients had an infection documented within 3 months before the diagnosis, mostly upper respiratory tract infections (76%). We found no significant relationship between Group 1 and Group 2 for the presence of prodromal infection (p>0.05, **Table 2**). In total, 42 patients (37%) had DKA at diagnosis. At initial presentation, there was no significant difference in terms of frequency of DKA between the groups (p>0.05, **Table 2**). Forty-two (37%) of the patients had a documented honeymoon period. Although, in Group 1 patients had earlier onset of honeymoon period than that of Group 2 patients, there was no significant difference between Group 1 and 2 with respect to the onset of honeymoon period (p>0.05). However, when compared with patients who had no documented honeymoon period (n=73), the patients who had documented honeymoon period (n=42) had lower HbA1c levels at the onset of the disease (13.7±4.2% versus 11.6±1.9%, p= 0.007). Blood pH and HCO_3 values were higher in Group 1 than Group 2, but the difference was not significant **(Table 3).** While pH and HCO_3 values were not significantly different between Group 1 and 2 female patients, male ones showed significant difference for HCO_3 values between Group 1 and 2 (19.7±6.4 mEq/L versus 15.0±7.8 mEq/L, p=0.045). Mean plasma glucose concentration was 401±135 mg/dL and mean HbA1c level at diagnosis was 12.6±3.3%. There was no significant difference in both parameters between the groups **(Table 3)**. Hashimoto thyroiditis was diagnosed in 10 patients (9%). Nine of the patients were in Group 1 and one of them was in Group 2 (p>0.05). While most patients (67%) were positive for one or more diabetes-related autoantibodies, only 33% of the patients were in idiopathic group. A large percentage (90%, n=69) of Group 1 patients were positive for GAD65. Among the GAD65-positive patients, 15 were positive for both

ICAs and AAIs. When analyzing the results of GAD65 -positive and -negative patients, we did not find any significant difference for all parameters. There were also no significant differences between the patients who had IAAs-positive (n=27) and IAAs-negative (n=88) or ICAs-positive (n=43) and ICAs-negative (n=72) in terms of epidemiological, clinical, and laboratory findings.

Discussion. In this paper, we described epidemiological, clinical, laboratory characteristics, and investigated whether the additional information provided by these data would be useful in helping to predict different clinical course in children with DM 1A or DM 1B. From methodological perspective, our study is one of scarce reports in English literature dealing with epidemiological, environmental, and clinical patterns of childhood DM1 with special regard to autoimmunity. The incidence of DM1 varies with age. Mean age at onset of the disease was found different in several studies from different parts of the world. Our results showed that 84% of the patients were ≥5 years of age. In the present study, the distribution of age at onset of diabetes was found similar in patients with DM 1A and DM 1B. In European countries, there seems to be more males with DM1, whereas a female prevalence is observed in non-European countries.[17] There was a female predominance in our both groups, which reflect a non-European population. In the present study, the fact that males with DM 1A had significantly higher HCO_3 levels than idiopathic ones that suggests the disease process might be associated with sex-dependent unknown factors. Breast-feeding or lack of it gives rise to one of the earliest exposures to the environment. There are many studies examining the diabetes risk with respect to a breast-feeding time of longer or shorter than 3 months.[13,18-20] However, different studies showed that breast-feeding or early introduction of cow's milk is unlikely to be among major factors that determine islet autoimmunity for DM1.[13,20,21] The present study demonstrates that, there is no significant association between breast-feeding time or early introduction of cow's milk and autoimmunity in the disease. Consanguinity rate in our country is estimated to be between 15% and 20%. While in our study, consanguinity between parents was 11%, Kandemir et al[22] reported the rate to be 24% of their diabetic patients who come mostly from the Central Anatolia region. Furthermore, our data provides no consistent evidence that the family history of DM1 or DM2 was associated with DM 1A or DM 1B. Nobody had a diabetic mother, and 5% of our patients had a diabetic father. Zalloua et al[23] reported a delay in the onset age of DM1 in patients with immediate family history of DM2, whereas no delay was observed in those with history of DM1. Also, their data did not show the presence of DM2 in parents that increases the risk for DM1 in their children. It was previously observed that the risk of developing diabetes is higher in offspring of fathers than of mothers with DM1.[24] The reasons for sex differential are unclear. Lorenzen et al[25] reported that the cumulative risk of DM1 up to 30 years was found to be significantly decreased in maternal offspring compared to paternal offspring, only if patients were diagnosed with DM1 before birth of the offspring. Several studies have suggested a relationship between month of birth and the risk of developing diabetes,[11,26,28] although other studies have failed to confirm such a relationship.[28,29] In our study, no significant correlation was found in birth seasonality in both groups. However, Samuelson et al[11] found that more children with diabetes than expected were born during summer, in agreement with Rothwell et al.[27] On the other hand, it was reported that there was a clear seasonal variation in the diagnosis time of diabetes. We found that more children with DM1 were diagnosed during the winter months, and the present study clearly confirmed seasonal variation in the diagnosis of diabetes. Moreover, autumn and winter were the dominant seasons in Group 1, however, it was statistically insignificant. In agreement with our finding, Hathout et al[30] found that winter was the predominant season for diagnosis of DM1. Seasonal factors could serve not only as a precipitating mechanism just before diagnosis, but also initiating or promoting mechanisms very early in the disease process. There are also seasonal variations in several immune variables, which could imply that some immunological abnormalities could be explained by seasonality. It has also been suggested that seasonal variation in nutrition or infections might influence pregnant mothers and their fetuses, or the baby during its early life.[11] In the present study, no significant correlation was found between presence of prodromal infection and risk for autoimmunity. On the other hand, although no immunoglobulin levels against viruses were measured in this study, most of our patients had upper tract infection in the prodromal period. The role of viral infections in the pathogenesis of DM1 has been supported by substantial new evidence suggesting that one virus group, enteroviruses, may trigger the beta-cell damaging process in a considerable proportion of patients.[30] Breast milk protects against enteric infections; enteric infections in turn could increase immunity to dietary antigens by increasing intestinal permeability. It was also suggested that there was an alteration in gut mucosal immune functions in genetically susceptible individuals.[31] In the present study, the DKA frequency at presentation was 37%. Although

it was not significant, this ratio was slightly higher in Group 2. Higher ratio of DKA at presentation may be a result of more severe insulin deficiency in these patients. The clinical data, including a trend for lack of a honeymoon period, suggest a more rapid or severe beta-cell destructive process in younger children. Although we could not perform any special pancreatic study, HbA1c levels were found to be significantly higher in patients with a lack of honeymoon period. On the basis of this finding, we suggest that lower HbA1c levels at presentation would be a forerunner for honeymoon period. Autoimmune thyroid disorders are common in patients with DM1. Approximately 22% of patients have thyroid antibodies.[5] In our study, Hashimoto thyroiditis was diagnosed in 10 patients (9%) and the ratio was higher in immune-mediated patients (11%), although the difference was not statistically significant. Autoantibodies against beta-cell antigens are detected in 85-98% of newly diagnosed children, furthermore, DM 1A is reported more common in whites and DM 1B is more common in non-whites.[5,32,33] In the present study, lower frequencies of diabetes-related autoantibodies at diagnosis of DM1 can be a result of rapid beta-cell destructive process. We did not follow their titer longitudinally. If we tested these antibodies, we might detect them in the later period. Komulainen et al[34] showed that there was a correlation between titer levels and the rate and intensity of beta-cell destruction, whereas other data provide evidence to the contrary.[11] Ziegler et al[35] reported that IAAs were the most often detected autoantibodies measured. They suggested that IAAs detection often preceded that of other autoantibody markers. On the other hand, GAD65 was the most detected islet autoantibody in our study. Autoimmunity and beta-cell death could be related to other environmental or genetic factors apart from the exposures considered in the present study. Due to lack of genetic analysis and control children, presence of fewer patients in Group 2, and retrospective nature of the study as limitative factors in our study, further researches are necessary to firmly establish the etiological factors and specific characteristics of the subtypes of DM1.

In conclusion, our study points that, there is no significant difference between DM 1A and DM 1B with respect to epidemiologic features and clinical presentation, suggest that these investigated factors does not play a major role in creating a disease-initiating and accelerating effect, or in the development of islet autoimmunity. However, determination of GAD65 and HbA1c levels may ensure some useful information regarding clinical course.

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