

HUMORAL IMMUNITY AND METABOLIC MARKERS IN THE INFANTILE POPULATION WITH DIABETOGENIC RISK. PRELIMINARY RESULTS

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REZUMAT

Scop: Identificarea copiilor cu risc de a dezvolta diabet zaharat insulinodependent (DZ tip 1). **Material și metode:** Lotul studiat include 41 de copii (vârsta între 3 luni și 18 ani) repartizați în 4 grupe de studiu: 32 de copii (frații copiilor cu DZ tip 1) au fost incluși în grupul A, 5 copii investigați pentru alterarea glicemiei a jeun au format grupul B, 1 sugar în vârstă de 3 luni provenit din mamă diabetică a reprezentat grupul C, 3 cazuri cu DZ tip 1 la debut investigați înainte de instituirea terapiei insulinice au reprezentat grupul D. La toate cazurile s-a determinat: glicemia a jeun, hemoglobina glicozilată (HbA_{1c}), colesterolul, trigliceridele, HDLc. În același timp s-a determinat markerii autoimunității umorale prin determinarea titrului anticorpilor anti decarboxilaza acidului glutamic (antiGAD), anticorpilor anti insule pancreatice (anti ICA). În grupurile A, B și C s-au considerat pozitive titrurile pentru percentila 97 și în grupul D pentru percentila 95. Dintre markerii metabolici am determinat concentrația peptidului C, cu valori normale între 0,5-3 ng/ml. **Rezultate:** În lotul A: 3 copii (9,37%) au fost GAD (+) și ICA (+), 23 cazuri (71,87%) au fost GAD (+) și ICA (-), iar 6 cazuri (18,75%) au fost GAD (-) și ICA (-). Peptidul C a fost în limite normale la cele 6 cazuri GAD (-) și ICA (-), și cu valoare mai mică de 0,5 ng/ml la cele 3 cazuri GAD (+) și ICA (+). La cele 23 cazuri GAD (+) și ICA (-) valorile peptidului C au fost atât în limite normale, cât și în afara intervalului normal. HbA_{1c} a fost în limite normale la toate cazurile. În lotul B toate cele 5 cazuri au fost GAD (+) și ICA (-), iar HbA_{1c} a fost normală. Sugarul provenit din mamă diabetică (lot C) a fost GAD (-) și ICA(-) cu o valoare HbA_{1c} = 5,3% și peptid C = 2,077 ng/ml. Lotul D (3 copii la debutul DZ tip 1) au fost GAD (+) și ICA (-). Peptidul C a fost în limite normale la 2 cazuri, iar al treilea a prezentat scăderea secreției de peptid C (0,191 ng/ml). HbA_{1c} a depășit valoarea de 12% la toate cele 3 cazuri. **Concluzii:** Anticorpilor anti GAD par să aibă legătură cu debutul DZ tip 1. Scăderea peptidului C pare să se coreleze numai cu prezența concomitentă a anticorpilor anti GAD, cât și a ICA.

Cuvinte cheie: autoimunitate, diabet zaharat tip 1, predicție, copil

ABSTRACT

Aim of the study: To identify the subjects at risk to develop of insulin dependent (type 1) diabetes mellitus (DM) in childhood. **Material and methods:** The study group included 41 children, aged 3 months - 18 years divided in 4 subgroups: 32 children (siblings of the children with overt type 1 DM) were included in group A, 5 children examined for impaired fasting glucose, formed group B, 1 toddler of 3 months from a diabetic mother represented group C and 3 cases with overt type 1 DM at onset, investigated before initiating insulin therapy, constituted group D. In all cases we determined: fasting glycemia, glycated hemoglobin (HbA_{1c}), total cholesterol, triglycerides, HDLc. Concomitantly, we determined markers of humoral autoimmunity by measuring the titer of anti-glutamic acid decarboxylase antibodies (anti GADA) and the antibodies against antipancreatic islet cells (ICA). In groups A, B and C we considered positive the titres above 97 percentile, while in group D above 95 percentile. Of the metabolic markers we used the evaluation of C peptide concentration, with normal ranges between 0.5-3 ng/ml. **Results:** In group A: 3 children (9.37%) were positive for anti GAD as well as for ICA, 23 (71.87%) were positive only for anti GAD and negative for ICA, and 6 cases (18.75%) were negative for anti GAD as well as for ICA. C peptide concentration was normal in all 6 cases negative for anti GADA and ICA and below minimal value (lower than 0.5 ng/ml) in all 3 cases positive for anti GADA as well as for ICA; in all 23 cases positive only for anti GADA the values of C peptide were either within or outside the normal range. In all cases from group A, HbA_{1c} was normal. In group B, all 5 cases were positive for anti GADA and negative for ICA, with normal HbA_{1c}. The toddler with diabetic mother (group C) was negative for both types of antibodies, with normal fasting glycemia, normal HbA_{1c} (5.3%) and normal secretion of C peptide (2.077 ng/ml). Interestingly, the 3 children with type 1 DM at onset (group D) were positive only for antibodies anti GAD (with titres above the upper limit of normal in all 3 cases), 2 of these having a titer 20 fold higher, but with a normal secretion of C peptide. The third case had a decreased secretion of C peptide (0.191 ng/ml). The HbA_{1c} in all these 3 cases exceeded 12%. **Conclusions:** The decreased secretion of C peptide seems to correlate only with the concomitant presence of anti GAD as well as ICA antibodies. Anti GAD antibodies seem to be significantly related with the onset of type 1 DM, fact proved by the significant titer in 2 of the 3 cases with IDDM at onset.

Key Words: autoimmunity, type 1 diabetes mellitus, prediction, child

INTRODUCTION

Insulin-dependent type 1 diabetes mellitus (type 1 DM) occurs consecutively to an irreversible destruction of the pancreatic insulin secreting beta cells. The destructive process is immune (in most cases), occurs in genetically predisposed subjects, is induced and sustained by the intervention of multiple environment factors and affects approximately 10% of the population.^{1,2}

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Currently, it is unanimously accepted that the short prediagnostic period in type 1 DM is the top of a huge iceberg, just partially explored by the modern immunogenetic studies. These studies prefigure a stadial evolution of a variable duration that may sometimes take more than 10-15 years.³ Within this interval the diseases passes through six evolutive stages: genetic predisposition, even if in more than 85% of the diabetic patients the specific history is negative, the intervention of a trigger factor of the autoimmune process, the autoimmune destruction of the beta cells, the impairment of the first phase of the insulin secretion, overt diabetes and the quasi-complete destruction of the beta pancreatic cells.⁴

These six phases might be reduced to three distinct phases: the first phase is represented by the genetic susceptibility, consisting in the existence of the HLA markers predisposing to the occurrence of type 1 DM. The DAISY study (Diabetes Autoimmunity Study in the Young), performed a decade ago, using a rapid screening method of the HLA aleles in newborns using cord blood, revealed the presence of a high risk HLA genotype (DRB1 03/DRB1 04, DQB1 0302) in 2.4% of the newborns.⁵

Presently it is accepted that type 1 DM in children is associated with HLA DR3, DQB1 0201 and DR4, DQB1 0302 and the decreased frequency might explain the reduced incidence of diabetes mellitus in some countries like Romania.^{6,7} Although the HLA antigens are actually the only well-known susceptibility markers, there are numerous data resulting from the twin studies showing that genetic susceptibility is only partially controlled by HLA alleles.

The second phase consists in the intervention of various environmental factors: climate (temperature), viral infections, the exposure to cow-milk proteins, vitamin D and/or nitrates consumption etc.).⁸ These factors could modify the structure of the proteins composing the beta pancreatic cell, thus, these might become antigens.

The third pathogenic phase is represented by the inflammatory immune response of the Langerhans islands, expressed through insulinitis. Within this period, immunological markers including antiinsular cytoplasmic antibodies (ICA), antiinsulin antibodies (IAA) and antiGAD 65 antibodies might be found in the serum. Progression to type 1 DM is associated with intramolecular epitope spreading to disease-specific epitopes localized in the middle region of glutamic acid decarboxylase 65 (GAD65).⁹

Detection of the antibodies in serum is of

significant importance for diagnosis. Increased titer of antiinsular antibodies (ICA) is predictable for type 1 DM, prior to disease onset, as showed in first-degree relatives of the diabetic patients: 8 to 10% of those presenting high titers of ICA progress to DM within one year.

AIM OF THE STUDY

To identify the subjects at risk to develop of insulin dependent diabetes mellitus (type 1 DM) in childhood.

PATIENTS AND METHOD

The studied group included 41 children, aged between 3 months and 18 years divided in 4 groups of study:

- Group A: 32 children (siblings of the children with overt DM);
- Group B: 5 children examined for impaired fasting glycemia;
- Group C: 1 toddler of 3 months from diabetic mother;
- Group D: 3 cases with overt type 1 DM at onset, investigated before initiating insulin therapy.

In all cases, we determined: fasting glycemia, glycated hemoglobin (HbA_{1c}), total cholesterol, triglycerides, HDLc. (Fig. 1) In the same time we determined markers of humoral autoimmunity by measuring the titer of anti-glutamic acid decarboxylase antibodies (anti GADA) and anti-pancreatic islet cells antibodies (ICA) using a radioimmunological method.

The threshold for antibody positivity was considered the 97th percentiles in groups A, B and C, and the 95th percentile in group D. From the metabolic markers we used the evaluation of C peptide concentration, measured using RIA method, with normal ranges between 0.5–3 ng/ml.

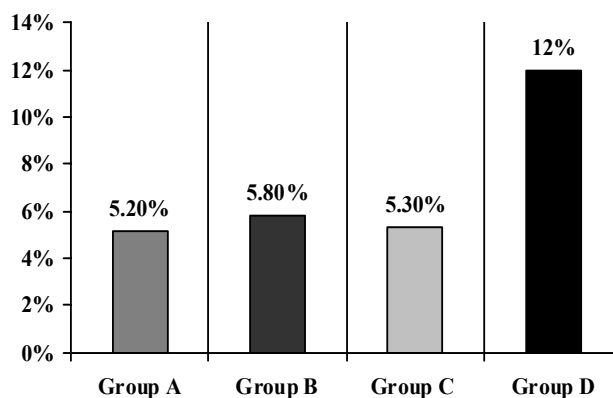


Figure 1. HbA_{1c} in the study subgroups (upper normal range: 5.7%).

RESULTS

In group A: three children (9.37%) were positive for anti GADA as well as for ICA, 23 (71.87%) were positive only for anti GADA and negative for ICA, and six cases (18.75%) were negative for anti GADA as well as for ICA. (Fig. 2)

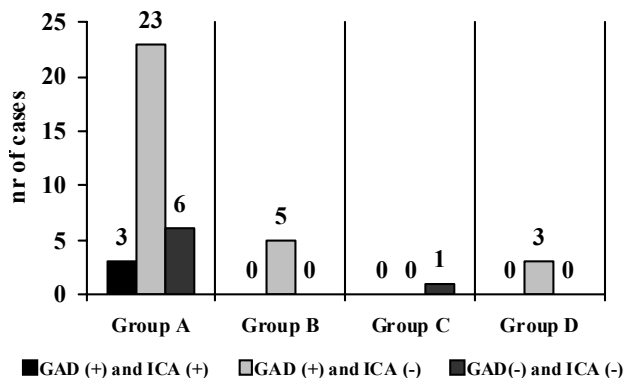


Figure 2. Distribution of study subjects depending on antibody positivity.

In group B, all five cases were positive for anti GADA and negative for ICA, with normal HbA_{1c}.

The toddler with diabetic mother (group C) was negative for both types of antibodies, with normal fasting glycemia, normal HbA_{1c} (5.3%) and normal secretion of C peptide (2.077 ng/ml).

In group A, C peptide concentration was normal in all six cases negative for anti GADA and ICA and below the minimal value (lower than 0.5 ng/ml) in all three cases positive for anti GADA as well as for ICA. (Table 1) In the 23 cases positive only for anti GADA, the values of C peptide were either within or outside the normal range. In all cases from group A, HbA_{1c} was normal.

Table 1. C peptide concentration in the study groups.

Group	C peptide value (normal: 0.5–3 ng/ml)		
	<0.5 ng/ml	0.5–3 ng/ml	>3 ng/ml
Group A: GAD (+) ICA (+)	3 cases	-	-
GAD (+) ICA (-)	5 cases	15 cases	3 cases
GAD (-) ICA (-)	-	6 cases	-
Group B: GAD (+) ICA (-)	-	5 cases	-
Group C: GAD (-) ICA (-)	-	1 cases	-
Group D: GAD (+) ICA (-)	-	3 cases	-

Interestingly, the three children with type 1 DM at onset (group D) were positive only for antibodies anti GAD (with titers above the upper limit of normal in all three cases, two of these having a titer 20 fold higher,

but with a normal secretion of C peptide. The third case had a decreased secretion of C peptide (0.191 ng/ml). The HbA_{1c} in all these three cases exceeded 12%.

DISCUSSION

Long-term follow-up of the first-degree relatives with high ICA titer suggested that the beta cell destruction run through a linear progressive process; often the destruction ratio presents variations between individuals, in some subjects being so slow that those would not develop overt type 1 DM along lifetime. The importance of ICA, GAD65 and IAA antibodies determination seems to be real in order to identify the subjects that are going to be included in prospective studies for the prevention of type 1 DM. The presence of autoimmune markers in association, both in the general population and in subjects belonging to groups with increased risk for type 1 DM, increases the probability for developing this disease.⁴

The Seattle Family Study used the combination of the autoimmunity markers with the metabolic ones and concluded that the first-degree relatives of type 1 DM patients, positive for ICA and anti-GAD, or with reduced first phase of the insulinic response, have an increased diabetogenic risk.¹⁰

Once the destructive autoimmune process is released, the lymphocytes, NK cells, macrophages and mastocytes are stimulated. These deliver cytokines such as interleukin 1 (IL-1), interleukin 4 (IL-4), alfa tumor necrosis factor, gamma interferon, that seem to be responsible for the aggression of the beta pancreatic cells. Experimentally, a significant impairment of the immune response IL-4 associated with IgG 4 towards tetanoid anatoxin was found in children with prediabetes.¹¹ It should be mentioned that the presence of significant concentrations of one or many immunological markers in the serum of diabetic patients is higher (getting up to 90%) in the immunologically active period that precedes the occurrence of the overt clinical disease and decreases progressively later on. Sometimes, also in healthy individuals, significant concentrations of diabetogenic antibodies might be detected and it might last years before the occurrence of the overt diabetes or even without developing the disease ($\approx 5\%$ of the general population).

The results of our study group seem to confirm the theory suggesting that genetic predisposition plays an important role in developing DM. This affirmation is sustained by group A (siblings of DM children) who were positive for antiGAD antibodies in a higher

number (26 cases).

The determination of the genetic structure (HLA antigens) in these cases, representing the second phase of the initiated study, could find the cases at risk to develop DM in near or farther future.

CONCLUSIONS

The decreased secretion of C peptide seems to correlate only with the concomitant presence of anti GAD as well as ICA antibodies. Anti GAD antibodies seem to be significantly related with the onset of type 1 DM, demonstrated by the significant titer found in two of the three cases at IDDM onset.

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