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Nilsson, Charlotta

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

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Short Report: Pathophysiology

Prevalence of zinc transporter 8 antibodies in gestational diabetes mellitus

J. Dereke[1], C. Nilsson[1,2], M. Landin-Olsson[1,3] and M. Hillman[1]

[1]Department of Clinical Sciences, Lund University, [2]Department of Pediatrics and [3]Department of Medicine, Helsingborg Hospital, Sweden

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Abstract

Aims Gestational diabetes mellitus affects approximately 7% of all pregnant women. Some of these women develop autoantibodies that are generally characteristic of Type 1 diabetes. Autoantibodies targeting glutamic acid decarboxylase and tyrosine phosphatase-like protein are the most frequently reported. A recently identified autoantigen in Type 1 diabetes is zinc transporter 8. Some reports suggest that the frequency of zinc transporter 8 antibodies is as high as glutamic acid decarboxylase antibodies in Type 1 diabetes and thus a good diagnostic marker for autoimmune diabetes. There are currently no reports of zinc transporter 8 antibodies in gestational diabetes. The aim of this pilot study was to investigate the frequency of zinc transporter 8 antibodies in patients at clinical onset of gestational diabetes mellitus.

Methods Subjects included in this pilot study were all diagnosed with gestational diabetes at Skåne University Hospital, Lund, Sweden, 2009–2010 (n = 193). Sera samples were analysed for antibodies using a commercial enzyme-linked immunosorbent assay according to the manufacturers' instructions.

Results We found that 19/193 patients with gestational diabetes, diagnosed in 2009–2010, were positive for at least one autoantibody. Glutamic acid decarboxylase was the most common single autoantibody (52.6%; 10/19), followed by zinc transporter 8 (21.1%; 4/19) and tyrosine phosphatase-like protein (15.8%; 3/19). Combinations of two or more antibodies were rare (10.5%; 2/19).

Conclusions In this study, we found that zinc transporter 8 added 2.1% (4/193) of autoantibody positivity in women with gestational diabetes who were negative for glutamic acid decarboxylase and tyrosine phosphatase-like protein antibodies. Glutamic acid decarboxylase was still the most prevalent autoantibody in gestational diabetes, but, as zinc transporter 8 was present even in the absence of glutamic acid decarboxylase, this autoantibody could be an important independent marker of autoimmunity in gestational diabetes.

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Introduction

Diabetes-specific antibodies towards zinc transporter 8 (ZnT8) particularly target the carboxyl terminal domain of the protein. Two single nucleotide polymorphisms (rs13266634 and rs16889462) have been described affecting amino acid position 325 [1]. The most frequently targeted C-terminal epitope contains an arginine (R325), followed by tryptophan (W325) and glutamine (Q325), respectively [2]. Screening for more than one epitope is recommended when searching for ZnT8 antibodies [3].

Several studies report the ZnT8 antibody as a good complement to glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like protein (IA-2) antibodies [3–6], in particular as a marker of adult-onset autoimmune diabetes [7] and Type 1 diabetes [8]. Reports of ZnT8 as the only detected autoantibody in people with diabetes range between 2.3 and 26% [4,5,8]. Some studies report frequencies of ZnT8 antibodies that are almost as high as GAD antibodies [4,8,9] at clinical onset of Type 1 diabetes, but often in combination with GAD and/or IA-2 antibodies. ZnT8 antibodies targeting epitopes containing arginine at amino acid 325 seem to be a stable marker, with prevalence peaking in late adolescence, before declining with increased age [8].

Approximately 7% of pregnant women worldwide are affected by gestational diabetes mellitus [10]. Gestational diabetes is associated with both fetal and maternal complications [11,12] and, although it shares many features with Type 2 diabetes, some patients will develop islet cell autoantibodies. These patients will have a significantly increased risk of

Correspondence to: Magnus Hillman. E-mail: magnus.hillman@med.lu.se

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developing Type 1 diabetes [13–15]. Identifying β-cell autoimmunity is also important, as the demand for insulin treatment during pregnancy is higher in young, autoantibody-positive women with gestational diabetes [14].

To our knowledge, there are currently no reports describing the prevalence of ZnT8 antibodies in gestational diabetes, which could identify ongoing autoimmunity in patients negative for GAD and IA-2 antibodies. The aim of this pilot study was to estimate the frequency of ZnT8 antibodies in patients with gestational diabetes and evaluate their importance as an autoimmune marker in gestational diabetes.

Patients and methods

The women included in the study were all the patients diagnosed with gestational diabetes after a 2-h 75-g oral glucose tolerance test at Skåne University Hospital, Lund, Sweden in 2009–2010 [n = 193]. Women previously diagnosed with gestational diabetes or with a family history of diabetes underwent a oral glucose tolerance test at the 12th week of gestation. Women without previous gestational diabetes or family history of diabetes were tested for gestational diabetes as a general screening at the 28th week of gestation. A 2-h capillary blood glucose value > 10.0 mmol/l was used as the diagnostic criterion for gestational diabetes. The mean age was 33.0 ± 5.5 years and none of the patients were on insulin treatment at the time of sample collection.

Blood samples were collected into serum tubes at Lund University Hospital and sent to the laboratory by ordinary mail. Sera was removed by centrifugation and stored in −70 °C until being analysed. The study was approved by the Ethical Committee at Lund University (849/2005).

Autoantibodies were analysed using a commercial enzyme linked immunosorbent assay (ELISA) from RSR Ltd (Cardiff, UK) according to the manufacturer's instructions, and cut-off levels for positivity were 15 U/ml for IA-2 and ZnT8 antibodies and 10 U/ml for GAD antibodies. The reported specificities for GAD, ZnT8 and IA-2 antibodies were 94, 99 and 100% and sensitivities were 90, 68 and 64%, respectively, in the Diabetes Antibody Standardization Program (DASP) 2010. The reported intra-assay variations for GAD, ZnT8 and IA-2 antibodies were 7.3, 6.0 and 3.6%, respectively. The interassay variations for GAD, ZnT8 and IA-2 antibodies were 5.7, 7.8 and 3.5%. C-peptide levels were analysed with a commercial ELISA from Mercodia (Uppsala, Sweden) according to the manufacturers' instructions. The detection limit of the assay was 15 pmol/l. Absorbance was measured at 450 nm in a BioHit BP808 plate reader (Biohit Health Care, Helsinki, Finland).

Normal distribution of data was examined using the D'Agostino–Pearson test. Mean and standard deviation were used when normality was accepted, and median followed by interquartile range in brackets were used when normality was rejected, if nothing else is stated. Spearman rank correlation was used to test for correlations and the Mann–Whitney U-test was used to test for differences between groups. P-values less than 0.05 were considered as statistically significant and the software MedCalc for Windows® v12.1.4 was used for statistical analyses (MedCalc Software, Mariakerkeb, Belgium).

Results

Autoantibodies

We found that 15/193 patients with gestational diabetes, diagnosed in 2009–2010, were positive for GAD and or IA-2 antibodies. When adding ZnT8 antibodies to the panel, the number increased to 19/193. GAD was the most common single autoantibody (52.6%; 10/19), followed by ZnT8 (21.1%; 4/19) and IA-2 (15.8%; 3/19) antibodies. Combinations of two or more antibodies were rare (10.5%; 2/19) (Fig. 1). Median autoantibody titres in U/ml, followed by (minimum–maximum) in patients regarded as autoantibody positive were 124 (18–2500) for GAD, 53 (23–1080) for ZnT8 and 55 (40–140) for IA-2 antibodies.

C-peptide

Median C-peptide levels did not differ between the group of autoantibody-positive (1.4; 1.0–1.6 nmol/l) and autoantibody-negative (1.1; 0.7–2.0 nmol/l) patients. There was neither any difference in C-peptide levels between GAD antibody-positive patients (1.4; 1.1–1.7 nmol/l) and patients positive for ZnT8 (1.2; 0.7–2.4 nmol/l) or IA-2 (1.5; 1.2–1.8 nmol/l) antibodies. We were also unable to detect any statistically significant correlation between C-peptide levels and GAD antibody titres (r_s = −0.02; P = 0.95) or IA-2 antibody titres (r_s = −0.02; P = 0.76) in our material. However, there was a weak trend towards high ZnT8 antibody titres and low C-peptide levels (r_s = 0.13; P = 0.07).

Age

The median age was similar in both the patients who were autoantibody positive (32.9 ± 4.0 years) and those who were autoantibody negative (33.0 ± 5.7 years). Neither were there any differences between patients who were positive for GAD (32.3 ± 2.7 years), ZnT8 (30.6 ± 3.0 years) or IA-2 (36.0 ± 5.2 years) antibodies.

Discussion

This pilot study is the first report of the frequency of ZnT8 antibodies in patients with gestational diabetes. ZnT8 antibodies were found in patients with gestational diabetes and most frequently without the coexistence of GAD and IA-2 antibodies.

A strength of the study is that the autoantibody assays used have reached excellent specificity in the Diabetes Antibody

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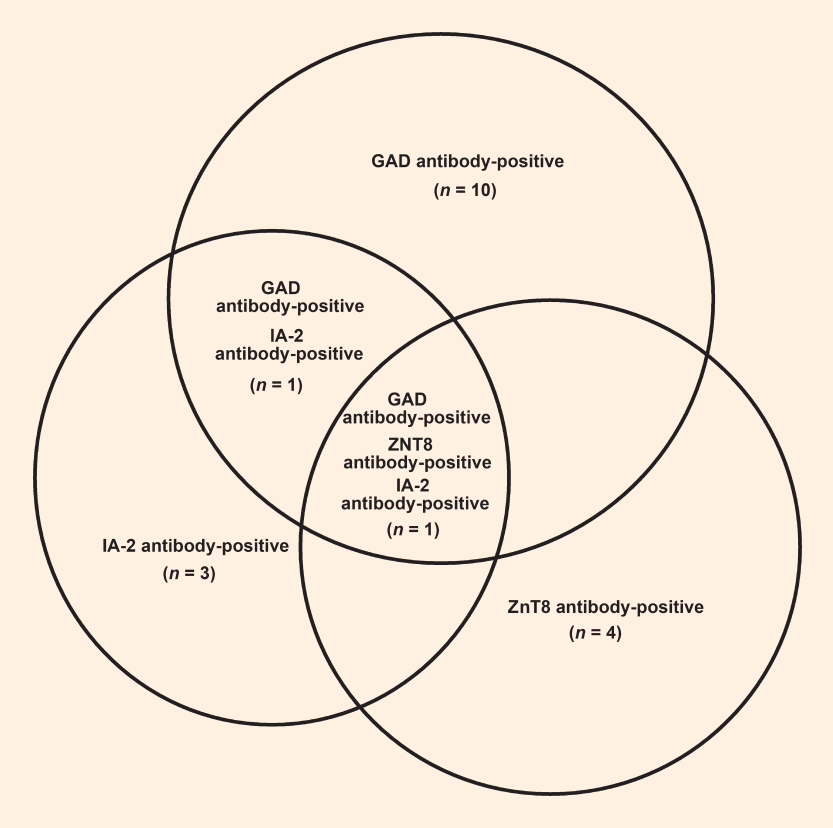

FIGURE 1 Venn diagram illustrating antibodies in patients with gestational diabetes. GAD antibodies were the most prevalent followed by ZnT8 and IA-2 antibodies. In 17/19 patients, only one autoantibody was prevalent.

Standardization Program 2010 evaluation and are easy to analyse. Another strength is that the ZnT8 antibody assay targets all polymorphic variants included in the C-terminal at amino acid position 325. By targeting both the arginine and tryptophan variants at position 325, the assay increases the sensitivity without lowering the specificity [3]. Limitations of the study are the relatively small number of subjects included and the lack of pregnant control subjects without diabetes.

Screening for GAD antibodies has previously been recommended in patients with gestational diabetes [15], as this is the most frequently found autoantibody in diabetes [14–16]. Even though this was also observed in our study, the incidence of autoantibody positivity in gestational diabetes increased from 6.2% (12/193) to 9.9% (19/193) by adding ZnT8 and IA-2 antibodies. It has been debated whether measuring autoantibodies other than GAD in gestational diabetes really adds prognostic information. Early studies showed that islet cell antibodies were predictive for the development of Type 1 diabetes in gestational diabetes [17], but the GAD antibody is part of the islet cell antibody. Neither IA-2 antibodies nor insulin autoantibodies have been considered as independent risk factors in gestational diabetes [14,17], but could ZnT8 antibodies add something of prognostic value?

In young first-degree relatives of patients with Type 1 diabetes, IA-2 and ZnT8 antibodies were associated with a fast progression toward disease within 5 years [6]. Even though the immune response is different in gestational diabetes compared with in Type 1 diabetes [18], the predictive value of ZnT8 antibodies is worth investigating. Also, combinations of antibodies have been shown to increase the risk of developing Type 1 diabetes in gestational diabetes [16] and all major autoantibodies should therefore be included in the screening process.

In young patients with acute-onset Type 1 diabetes, the prevalence of ZnT8 antibodies has been reported to be almost as high as GAD antibodies [8] and, in patients with latent autoimmune diabetes in adults (LADA), in the same range as IA-2 antibodies [7]. In these studies, ZnT8 antibodies were frequently found in combination with GAD and/or IA-2 antibodies. However, based on our observations, we suggest that ZnT8 antibodies could be an important marker for islet autoimmunity, independent of GAD or IA-2 antibodies.

Future research should aim to focus on the importance of the ZnT8 antibody as a marker in gestational diabetes, its use as a predictor of Type 1 diabetes post-partum and its relevance in combination with GAD and IA-2 antibodies. It could also be of importance to investigate ZnT8 antibody titres in autoantibody-negative pregnant women without diabetes. As the ZnT8 antibody has only been recently identified, such data will be of major importance when interpreting ZnT8 antibody titres in

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women with gestational diabetes and its progression to manifest diabetes.

In conclusion, by adding ZnT8 antibodies to IA-2 and GAD antibodies, the number of autoantibody-positive patients increased from 7.8 to 9.9%. Importantly, our pilot study demonstrates that women with gestational diabetes mellitus may be positive for ZnT8 antibodies, even in the absence of other markers of islet autoimmunity.

Funding sources

None.

Competing interests

Nothing to declare.

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