Gestational Diabetes Mellitus- Future risk for mother and child

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Clinical use of C-peptide and β-cell specific autoantibodies during gestational diabetes mellitus

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Abstract

Gestational diabetes mellitus (GDM) confers a risk for developing type 2 diabetes later in life, but the risk of developing type 1 diabetes is also increased. In this study we have evaluated the clinical use of C-peptide and β-cell specific autoantibodies during pregnancy with GDM as predictors for later development of diabetes.

C-peptide levels were measured 2 hours after glucose intake in pregnancies with GDM during 2006–2008 (n=281). The mother s age and first weight during pregnancy, birth weight of the newborn and postpartum development of diabetes in the women were noted from their records. Between 1995–2008, 669 women developed GDM and were tested for glutamic acid decarboxylase antibodies (GADA) and tyrosine phosphatase antibodies (IA-2A); 34 women (5%) were found positive for at least one autoantibody.

The incidence of diabetes was significantly higher ($p<0.001$) among women with positive autoantibodies (5/12) compared to women without autoantibodies (21/266) during 2006–2008. When comparing stimulated C-peptide during GDM between women who later developed diabetes and those who did not, there was no significant difference. Among the 34 women who were autoantibody positive during their GDM between 1995–2008, 50% (n=17) had developed type 1 diabetes, and an additional five had impaired fasting glucose or impaired glucose tolerance.

In conclusion, stimulated C-peptide values were of no use in women with GDM regarding prediction of future diabetes. Analysis of GAD antibodies during GDM is recommended, due to a high risk of type 1 diabetes after delivery. A structured follow up of all women with GDM ought to be considered.

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Key words

gestational diabetes; C-peptide; β-cell autoantibodies

Introduction

Gestational diabetes mellitus (GDM), depending on the population studied, affects 1–14% of all pregnant women.[1] It is well known that women with GDM have a substantial risk of developing type 2 diabetes (T2DM) later in life,[2] but the risk of developing type 1 diabetes (T1DM) is also increased.[3] C-peptide is a stable marker for endogenous insulin production, and assays are widely used for evaluation of the β-cell reserve[4] and differential diagnosis between T1DM and T2DM.[5] Determination of C-peptide is preferable compared to insulin measurements for two reasons. First, the β-cell production of insulin can be estimated despite ongoing treatment with exogenous insulin. Second, insulin is rapidly extracted by the liver with individual variation, which makes insulin levels inferior in estimating actual β-cell production.[6]

β-cell specific autoantibodies such as glutamic acid decarboxylase antibodies (GADA)[7] and tyrosine phosphatase antibodies (IA-2A)[8] are useful for identifying autoimmune diabetes. Analysis of GADA is preferable since it is the only non age-dependent autoantibody and 80% of patients with TIDM are positive for GADA at diagnosis,[9] which is higher than for IA-2A. Our previous study showed that among women with GDM, 6% had autoantibodies and 50% of these women developed T1DM during the follow up.[3]

The first purpose of this study was to evaluate C-peptide levels in women with GDM as a predictor for future development of diabetes. Our second purpose was to investigate the role of C-peptide in relation to other birth related factors in GDM patients, such as age, first weight during pregnancy of the mother and birth weight of the newborn. The third purpose was to

follow up our previous study of T1DM development in women with GDM and autoantibodies.[3]

Materials and methods

A 2-hour oral glucose tolerance test (OGTT) in the 28th gestational week is performed on every pregnant woman in our region in Sweden as a screening for GDM. Women with prior GDM and/or heredity for diabetes are already tested during the 12th gestational week. The 2-hour OGTT capillary plasma glucose value for defining GDM was >10.0mmol/L, or 9.0mmol/L for capillary blood glucose.[10] All women who developed GDM during 1995–2008 (n=669) were tested for the autoantibodies GADA and IA-2A. There were 34 women (5%) who were positive for at least one antibody. Their medical records were examined regarding later development of T1DM. The follow-up time for these autoantibody-positive women was between two and 15 years, with a median of nine years. The 385 pregnant women with GDM from our previous study[3] were included in this study.

C-peptide levels were also measured in all pregnancies of women with GDM during 2006–2008 (n=281). Three women were diagnosed twice with GDM during this time period. Only their first pregnancy was used for comparisons. The medical records of all pregnant women with GDM during 2006–2008 were examined regarding age, first weight of the mother during pregnancy and birth weight of the newborn. The incidence of postpartum development of diabetes was collected from their current medical records. All women with GDM are followed at our clinic with an annual OGTT during the first two years and thereafter five years postpartum. If they develop T2DM they are transferred to their health care centre. In women who are autoantibody positive during their pregnancy, the first OGTT is performed three months postpartum and those who develop T1DM are followed at our department of endocrinology.

The samples for C-peptide were taken after a 2-hour OGGT and were analysed by radioimmunoassay, using a commercial kit (Euro-Diagnostica, Malmö, Sweden). The reference range was only defined for fasting condition and was 0.25–1.0nmol/L. The assay had a detection limit of 0.13nmol/L.

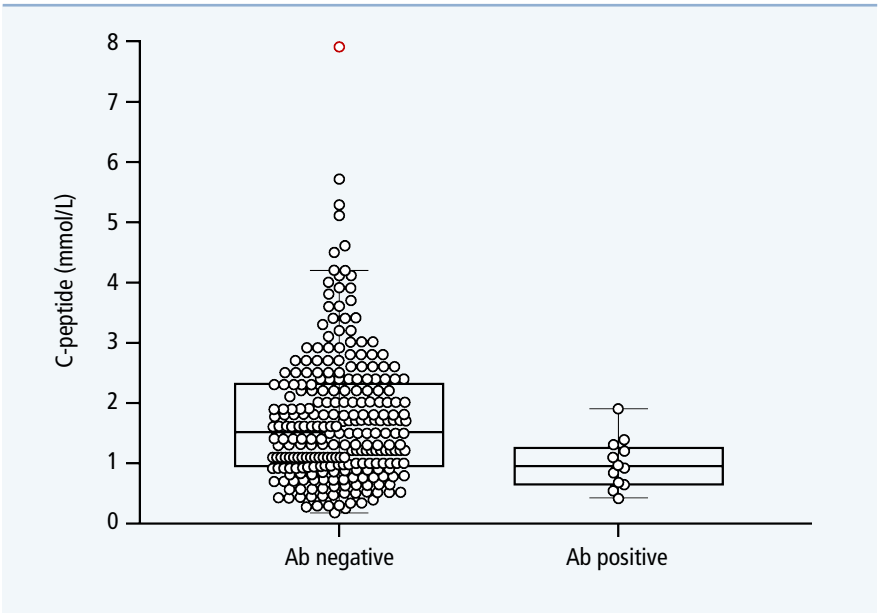

Figure 1. Comparison of stimulated C-peptide levels in GDM women during 2006–2008 with (n=12) or without (n=261) autoantibodies

GADA and IA-2A were analysed in similar radioimmunoprecipitation assays[7,8] with *in vitro* translated human GAD65 and IA-2A respectively, both antigens labelled with ^{35}S-methionine. An index calibrated to a positive and negative standard expressed the levels. GADA indexes <0.08 and IA-2A indexes <0.05 were defined as negative and represented values below the 97.5th percentile. The GADA assay had a sensitivity of 70% and a specificity of 100% and the corresponding figures for IA-2A assay were 50% and 100%, respectively, when tested in the Diabetes Antibody Standardization Program.[11] Since 2006, assays for GADA (RS-GDE) and IA2A (RS-IA2E) were supplied by RSR® Ltd, Cardiff, UK, and were performed according to manufacturer's instructions.

The study was approved by the Ethical Board at Lund University, Sweden.

Statistical analysis. Normal distribution was tested with D'Agostino-Pearson test. The normally distributed results are presented as mean ± SD and the t-test was used for comparisons between groups. The non-parametric results are presented as median (range) and the Mann-Whitney test was used for comparisons between groups. The frequencies are presented as numbers (percent) and were compared using Chi-square test. Correlations were tested using Spearman's rho (rs) correlation test. A p-value of <0.05 was considered significant. A Kaplan-Meier Survival Analysis was used to examine the time from GDM with autoantibodies until the development of T1DM. The programs Statistical Package for the Social Sciences for Windows (version 17.0) and MedCalc® for Windows (version 12.0.3.0) were used for the analysis.

Results

C-peptide in relation to later development of diabetes. The stimulated C-peptide levels were compared in GDM women during 2006–2008 with (n=12) or without (n=261) autoantibodies. The median C-peptide level was significantly higher in the group without autoantibodies 1.5 (0.2–7.9) than in the group with autoantibodies 0.9 (0.4–1.9) (p=0.007; Figure 1). In five cases, C-peptide levels were missing from the medical records.

The incidence of diabetes was significantly higher (p<0.001) among women with positive autoantibodies (5/12) compared to women without autoantibodies (21/266) during 2006–2008.

When comparing stimulated C-peptide during GDM between women who later developed diabetes and those who did not, there was no

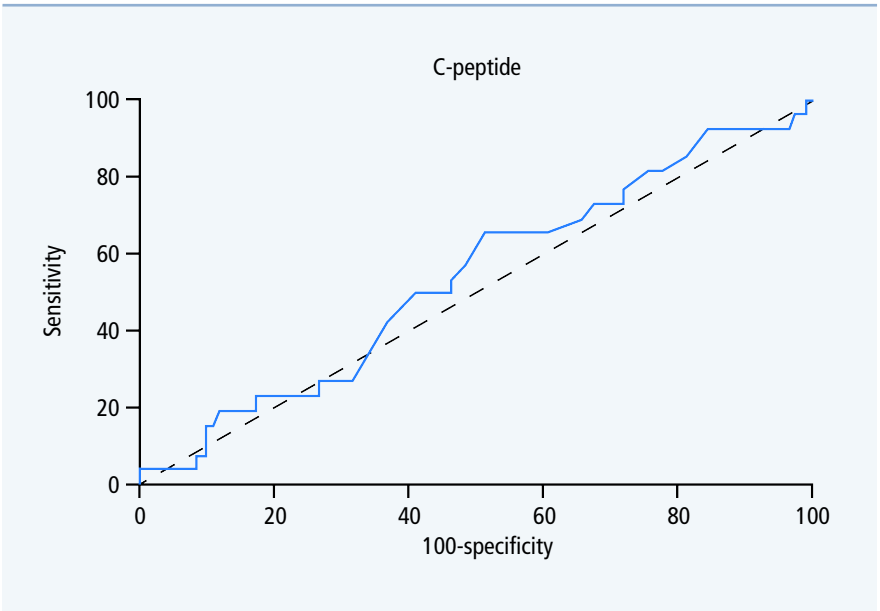

Figure 2. ROC curve comparing stimulated C-peptide during GDM between women who later developed diabetes and those who did not

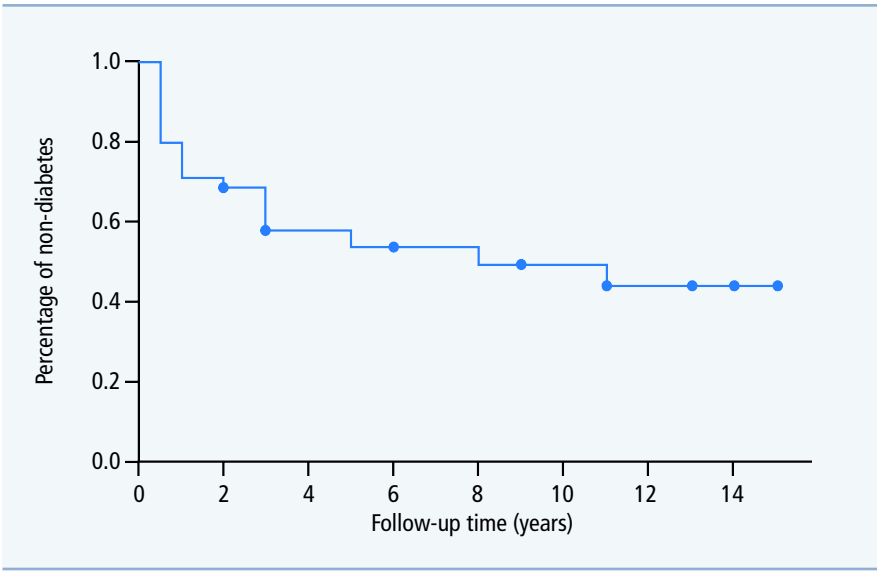

Figure 3. Kaplan-Meier Survival Analysis showing the time from GDM until development of type 1 diabetes in 34 autoantibody positive women

significant difference. This is illustrated by a ROC curve shown in Figure 2 where no distinct C-peptide value can be identified to differ between those who developed diabetes and those who did not.

C-peptide correlations in women with GDM. C-peptide levels correlated significantly with the women's first weight during pregnancy (rs=0.3; p=0.0003). The median first weight during pregnancy of women without autoantibodies was 68.0kg (44.4–150.0; n=196) and of women with autoantibodies 56.5kg (48.0–105.0; n=8); (p=NS). There was no correlation between the birth weight of the newborn or the mother's age at delivery to the C-peptide levels. The mean birth weight of the newborn was 3408.7±541.1g in the group without autoantibodies (n=213) during their GDM and 3473.1±519.3g in the autoantibody positive group (n=8); (p=NS). The mothers' median age at delivery was 33 (17–44) years among the women without autoantibodies (n=266) and 33.5 (20–42) years among the autoantibody positive women (n=12); (p=NS).

Follow-up of the autoantibody positive women with GDM. There were 34 women (5%) who were positive for at least one antibody. Their medical records were examined regarding later development of T1DM. The follow-up time for these autoantibody-positive women was between two and 15 years, with a median of nine years.

From the medical records at follow up of the 34 women who were autoantibody positive during their GDM, we found that 17 (50%) had developed T1DM. The median follow up for the autoantibody positive women was nine (two to 15) years. A Kaplan-Meier Survival Analysis showing the time from GDM until development of T1DM in these 34 women is presented in Figure 3. The medical records at follow up also showed that five of the 34 women had impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) at least once after their pregnancy with GDM and persisting positive autoantibodies.

In addition, six of the 34 women were pregnant again with GDM and positive autoantibodies. Consequently, a complete normal glucose tolerance was found only in 18% of these women with previous autoantibody positive GDM.

The majority of the autoantibody positive women had GADA (94.1%, 32/34) and about one-third had IA-2A (29.4%, 10/34).

Discussion

GDM entails a great risk for T2DM later in life. In this study, we wanted to explore the clinical use of C-peptide and β-cell specific autoantibodies as predictors for future development of diabetes. Our major finding was that C-peptide did not discriminate between women who developed diabetes during follow up and those who did not, while GAD antibodies were of significant value in identifying patients in risk of T1DM.

Since GDM poses such a high risk for later diabetes it is imperative to follow these women to avoid metabolic deterioration and enable intervention and early treatment. Because diabetes can occur several years after the pregnancy it is desirable to find a tool to quantify the risk in the individual woman. Known risk factors

such as age, ethnicity, obesity and family history of diabetes[12] are not sufficiently specific to allow an exclusion of women without or with a low risk. Levels of C-peptide correlated significantly with the women's first weight during pregnancy but otherwise no correlations with C-peptide levels were found. C-peptide levels were not able to predict future development of diabetes in the woman.

In this study, samples for C-peptide were taken 2 hours after an oral glucose intake one to two weeks after the screening OGTT in gestational week 28, when GDM was diagnosed, meaning that there was one trimester left before delivery. A stimulated C-peptide at this time is mainly a measure of the ability of the β-cells to respond to glucose and thereby reflecting the β-cell reserve. Later during pregnancy, with an increasing demand of insulin, the β-cells would be maximally strained and perhaps show signs of being exhausted. In this study, it would perhaps have been preferable to measure insulin resistance in the peripheral tissue, since insulin resistance has been shown to be a strong risk factor for future diabetes. It could have been done by analysis of plasma (p-) glucose and p-insulin in fasting samples for estimation of HOMA (homeostasis model assessment) index,[13] but the design of the study did not include collection of fasting samples. We could not define any level of stimulated C-peptide that distinguished between women who later developed diabetes and those who did not. A limitation of this study was the lack of C-peptide data on patients diagnosed with GDM before 2006.

The frequency of β-cell specific autoantibody positivity, mainly represented by GAD antibodies, was 5% in our study of women with GDM. We found that these autoantibodies were highly prognostic for later development of T1DM, which is a confirmation of our previous study.[3] In the two separate groups of autoantibody positive and negative women with GDM, the C-peptide levels were significantly lower in the antibody positive group, but the overlap was so great that the practical use of C-peptide to identify autoimmune diabetes was insufficient. C-peptide was therefore of very limited clinical usefulness and could not be used to replace testing of GADA.

We found that 50% of the women with autoantibodies during their GDM had developed insulin dependent diabetes during the study period and an additional five had IFG or IGT. Six women had a new GDM at the time of follow up and they had persisting autoantibodies. This meant that 82% of women with the presence of autoantibodies during previous GDM had an abnormal glucose tolerance at follow up. It is known that GADA positivity can precede and also persist after the diagnosis of T1DM.[14] It cannot therefore be excluded that even more of these women might develop T1DM in time.

Previous studies have shown that the rates of GADA positivity range from 0% in women with GDM in northern Italy[15] up to 10% among women with GDM in a German multicentre study.[16] The discrepancies could partly depend on different ethnic groups or different screening systems. In our study, the most frequent autoantibody was GADA since 32 of the 34 women with autoantibodies were positive for this. Even if the prediction of diabetes was high after screening with GADA, the autoantibody positive women will only account for 5% of all the patients with GDM and will not catch the main part at risk. GADA positivity is highly predictive for T1DM, but does not include the majority of women who develop T2DM. We still have no reliable marker for T2DM.

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Declaration of interests

There are no conflicts of interest declared.

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Key points

- Women who have had GDM should be offered a structured programme for follow up during several years, since the progression to manifest diabetes is higher than in all other risk groups
- GAD analyses should be performed in all women with GDM since a frequency of 5% is not negligible and the risk for type 1 diabetes is at least 50% among women with autoantibodies during GDM
- Commercial GADA ELISAs are easy to obtain and manage. The expense is relatively low and does not require advanced laboratory equipment
- Analysis of C-peptide should not be done by routine, since the analysis does not add any valuable information to this group of patients

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