Diabetic Complications Consortium

Application Title: Islet Autoantibodies in Diabetic Patients with Symptoms of Gastroparesis

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1. Project Accomplishments:

This study has successfully measured GAD-65 and c-peptide levels in a large number of patients with diabetic gastroparesis. This study suggests islet antibodies are prevalent in most diabetic patients with gastroparesis. This study found the presence of islet antibodies in both T1DM and T2DM with symptoms of gastroparesis. Although it is well known that the vast majority of patients with type 1 diabetes have autoantibodies, the fact that the proportion of patients with type 2 diabetes who were GAD positive was high is novel, suggesting autoimmunity may be playing a role in T2DM patients who develop gastroparesis, or at least symptoms of gastroparesis. This study also suggests that c-peptide levels may better divide patients into T1DM vs T2DM, then by patient or physician diagnosis.

2. Specific Aims:

While gastroparesis in type 1 diabetes mellitus (T1DM) patients is well recognized as a long term complication of diabetes mellitus, gastroparesis is being increasingly diagnosed in type 2 diabetes (T2DM). T1DM is felt to be autoimmune mediated, as it is associated with autoantibodies, particularly anti-glutamic acid decarboxylase-65 [GAD65] antibody. Our overall hypothesis is that T2DM who develop gastroparesis are more likely to have an autoimmune form of the disease, i.e. latent autoimmune diabetes in adults (LADA), more closely related to T1DM than those who do not. Through these studies on islet autoantibodies in diabetic patients with gastroparesis, we hope to gain insight in the phenotypic characteristics of this complication of diabetes - diabetic gastroparesis.

Specific aims:

Aim 1. Determine the prevalence of islet autoantibodies using anti-glutamic acid decarboxylase-65 [GAD65] antibody) in T1DM and T2DM patients with symptoms of gastroparesis. Compare the prevalence of islet autoantibodies in symptomatic T2DM patients with and without delayed gastric emptying.

Aim 2. Determine the gastroparesis phenotypic profile (gastric emptying times and gastroparesis symptoms as determined by the Gastroparesis Cardinal Symptoms Index [GCSI]) of T2DM gastroparesis patients having islet autoantibodies. Compare the gastroparesis symptoms and rate of gastric emptying of T2DM gastroparesis patients with islet autoantibodies to T2DM gastroparesis patients without islet autoantibodies and to T1DM with gastroparesis.

Aim 3. Determine the diabetic phenotypic profile (body weight, C peptide level, and glucose control measured by fasting glucose, and A1c) of T2DM gastroparesis patients having islet autoantibodies. Compare the diabetic phenotypic profile of T2DM gastroparesis patients with islet autoantibodies to patients with T2DM gastroparesis patients without islet autoantibodies and to T1DM with gastroparesis.

Methods:
This study used the data set of the NIH Gastroparesis Clinical Research Consortium Gastroparesis Registry. This is a multicenter registry involving eight centers. To enter the registry, patients with symptoms of gastroparesis underwent detailed history and filled out questionnaires about their symptoms, using the Gastroparesis Cardinal Symptom Index (GCSI). Patients also had a dedicated 4 hour gastric emptying test. Patients had blood drawing including glucose, HgbA1c, and samples of serum and plasma were saved. Diabetic patients in the NIH Gastroparesis Registry studies (GpR1 and GpR2) were profiled as to the presence of GAD-65 antibodies and c-peptide levels using their banked serum that was saved at their time of enrollment. The assay was run by the research laboratory at Quest Diagnostics.

Results:
The registry data set for this study contained 113 patients with T1DM and 90 patients with T2DM. All patients had symptoms suggestive of gastroparesis to enter the GpR; most had delayed gastric emptying. Delayed gastric emptying was present in 89 of the 113 (81.7%) T1DM and 58 of the 90 (66.7%) T2DM patients.

Characteristics of diabetic patients by diabetic type (T1DM vs T2DM)
T1DM patients, as compared to T2DM patients, were younger at time of diagnosis of diabetes (18 vs 40 years) and younger at time of diagnosis of gastroparesis (34 vs 48 years). At time of enrollment into the GpR, T1DM, as compared to T2DM patients, were younger, and had longer duration of diabetes (22 vs 12 years) and longer duration of gastroparesis (7 vs 4 years). Gastric emptying was more delayed in T1DM than T2DM (38 vs 21% retention at 4 hours). Delayed gastric emptying was present in 89 (81.7%) of T1DM and 58 (66.7%) of T2DM patients. T1DM patients had greater HgbA1c than T2DM. Islet autoantibodies (GAD>1.0 U/ml) were present in 62 (55.4%) of T1DM patients and 78 (86.7%) of T2DM. C-peptide was normal (>0.8 ng/ml) in 23 (21.7%) of T1DM and 83 (92.2%) of T2DM patients. Of GI symptoms, vomiting severity was greater in T1DM patients whereas early satiety was greater in T2DM.

Characteristics of diabetic patients by level of GAD-65.
Positive GAD-65 antibodies were present in 140 of the 203 diabetic patients: 62 of the 113 T1DM patients and in 77 of the 90 T2DM patients. Patients with positive GAD-65 antibodies were older, had a greater age at diabetes diagnosis, greater age at diagnosis of gastroparesis, but shorter duration of gastroparesis symptoms (5 vs 7 years). Patients with positive GAD antibodies had similar gastric emptying, HgbA1c, and symptoms of gastroparesis.

Characteristics of T1DM patients by level of GAD-65.
GAD antibodies were present in 62 of the 112 (55%) patients with T1DM. T1DM patients with positive GAD antibodies were more commonly black race and less likely white race.

Characteristics of T2DM patients by level of GAD-65.
GAD antibodies were detected in 78 of the 90 (86%) patients with T2DM. T2DM patients with positive GAD antibodies were less likely to be Hispanic. T2DM patients with positive GAD antibody had shorter duration of gastroparesis at enrollment. T2DM patients with positive GAD had similar duration of diabetes at enrollment but less duration of gastroparesis at enrollment (4

vs 7 years). T2DM patients with positive GAD antibodies had a trend to more delayed gastric emptying 22 vs 14% retention at 4 hours (p=0.24), higher HgbA1c levels (8 vs 7%; p=0.18).

Characteristics of diabetic patients by C-peptide levels
Low C-peptide levels were seen in 88 patients with T1DM and in 7 patients with T2DM. Patients with low c-peptide levels had younger age of onset of diabetes and onset of gastroparesis. Patients with low c-peptide levels were more likely to use insulin compared to those with normal c peptide levels (100 vs 58%). Patients with low c-peptide levels had greater HgbA1c and greater retention on gastric emptying.

Characteristics of T1DM patients by C-peptide levels
Low C-peptide levels were seen in 88 of the 113 T1DM patients. The age at diabetes diagnosis were younger for T1DM with low c-peptide than for T1DM with normal C-peptide. All patients with T1DM and low c-peptide were taking insulin, whereas 78% of patients with T1DM with normal C-peptide were.

Characteristics of T2DM patients by C-peptide levels
Low c-peptide levels were seen in 7 of the 90 T2DM patients. All 7 patients were taking insulin compared to 51.8% of those with normal c-peptide levels.

Summary of results
1.GAD-65 antibodies are present in both T1DM and T2DM patients with symptoms of gastroparesis.
2.In this series of patients with symptoms of gastroparesis, the prevalence of GAD-65 antibodies was higher in T2DM then T1DM. Some of this may be related to misclassification of T1DM vs T2DM by self-report. Presumably all of the T1DM patients had autoantibodies at diagnosis that disappeared over time.
3. Use of insulin for clinical care was used in all patients with low c-peptide levels as expected, but in only half of patients with normal c-peptide levels.
4. The diagnosis of T1DM by self-report of patients is not as reliable as diagnosis of T2DM. In this series, evidence for misdiagnosis of T1DM by using c-peptide levels were seen in 25 of 113 patients, whereas misdiagnosis of T2DM in 7 of 90.
5. The major and surprising finding of this study was that fact more than 80% of individuals with type 2 diabetes and gastroparesis were GAD-65+. Although studies are limited, previous work in patients with type 2 diabetes had found positive autoantibodies in only 5 to 10% of individuals diagnosed with type 2 diabetes.

Conclusions
This study suggests islet antibodies are prevalent in most diabetic patients with gastroparesis. This study found the presence of islet antibodies in both T1DM and T2DM with symptoms of gastroparesis. Although it is well known that the vast majority of patients with type 1 diabetes have autoantibodies, the fact that the proportion of patients with type 2 diabetes who were GAD positive was high is novel, suggesting autoimmunity may be playing a role in T2DM patients who develop gastroparesis, or at least symptoms of gastroparesis. This study also suggests that c-peptide levels may better divide patients into T1DM vs T2DM, then by patient or physician diagnosis.

Considerations for additional analysis

1.Compare the T2DM patients by gastric emptying test results: delayed gastric emptying vs normal gastric emptying (compares12 vs 78 patients)

2. Re-do tables using classification of type1 vs. type 2 diabetes based on C-peptide levels.

3.Multivariable analysis. GAD-65 antibody versus no GAD-65 antibody.

4. Confirm GAD-65 and c-peptide results by another reference laboratory. Consider also using IA-2A (insulinoma antigen autoantibodies)

We have a very challenging finding. The Type 1 pool has a very high proportion of negative anti-GAD (much more than expected) and also a larger than expected proportion with C-peptide levels considered normal levels or high, especially with long standing Type 1 diabetes. Type 2 diabetic pool has more anti-GAD positives than expected but the proportion of positive C-peptide is right. Possible explanations: 1) The clinical dx of patients at baseline is equivocal; 2) The assay is equivocal or, 3) a combination of both 1 and 2 or,

Possible solutions: 1) Show data to lab for their commentary on the unusual results - GAD65 should be positive in less than 5% of type 2 diabetes patients and be of very low titer. This antibody is sometimes used to distinguish between type 2 and 1. 2) Repeat GAD and c-peptide in different lab and if possible consider IA-2A (insulinoma antigen autoantibodies)

5.Controls for GAD-65: non Gp DM controls, non DM but Gp controls, non DM and non Gp controls. GAD65 may be a very strong risk factor for gastroparesis; this was found achalasia many years ago.

6.Re-examine type of diabetes and re-adjudicate after having more certainty of assay results the type of diabetes in each case.

3. <u>Publications:</u>

Planning submission of abstract to 2016 Digestive Disease Week. Abstract due December 1, 2016.