The effect of gluten-free diet on type 1 diabetes (T1D)

NCT02867436

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Trial design

This study is a prospective non-randomized intervention trial.

Participants

Study participants were recruited from children patients diagnosed with T1D at inpatient pediatric diabetes clinics at two centers in Prague, Czech Republic (University Hospital Prague-Motol and University Hospital Prague-Královské Vinohrady). Both centers are tertiary care hospitals that annually admit over 65 children patients with newly diagnosed T1D. The recruitment will last from January 2016 to January 2018. Subjects and their parents have the right to withdraw from the study at any time without giving the reason.

Eligibility criteria

Following inclusion criteria will be applied:

- T1D diagnosed according to the ADA criteria [1]
- age 4 – 17.99 years at onset
- positivity of a least one T1D associated antibody (IAA, anti-IA2, anti GAD65)
- presence of high risk HLA-DQB1*03:02-DQA1*03 or DQB1*02-DQA1*05 molecules
- no coeliac disease and negativity of anti-tissue transglutaminase antibodies
- body mass index below +2SD of the age and height standard
- no concomitant disease potentially influencing immune response or gluten sensitivity

Intervention

Subjects in the intervention group will follow strict gluten-free diet while receiving the recommended daily doses of macronutrients. Their meal records for three days prior to the visit will be required for each visit as well as a consultation with nutrition specialist. Adherence to the diet will be tested via the negativity of the gluten immunogenic peptide (GIP) in the commercial iVylisa GIP-S (Biomedal, S.L., Sevilla, Spain) ELISA kit. Stool samples will be tested for GIP presence every 3 months without especially notifying the subjects. Subjects in the intervention arm will receive financial reimbursement for the added cost of gluten-free edibles.

Study procedure

The caregivers of the subjects will sign a written informed consent with the study protocol prior to the enrollment in the first 30 days after T1D diagnosis.

All subjects will be hospitalized at T1D onset. During the hospitalization, a blood sample will be taken for the measurement of anti-GAD65, anti-IA2 and IAA, glycosylated hemoglobin (HbA1c) (performed by high liquid chromatography) and screening for coeliac disease (total IgA to rule out IgA deficiency anti anti-tissue transglutaminase antibodies analyzed by ELISA test). A stool sample will also be taken for further analysis of their gut microbiome content.

If the subjects fulfill the inclusion criteria, they will be offered to join the trial. The subjects and their parents will choose their study group freely and a mixed-meal tolerance test (MMTT) will scheduled between 4-8 weeks after T1D onset.

The MMTT is considered a gold standard for the measurement of the endogenous production of residual insulin in subjects with T1D [2]. MMTTs will be performed for all subjects at group allocation and at 6 and 12 months. All subjects will receive a standard liquid meal Ensure Plus (Abbott Laboratories BV, Zwolle, Netherlands) and their C-peptide levels will be measured at the baseline and each 30 minutes up to 150 minutes. All MMTTs will be performed after overnight fasting and children will receive only their evening dose of long-acting insulin analogue. The MMTT will be rescheduled if the subjects` capillary glucose is <3.9 mmol/L (<70 mg/dL) or >10 mmol/L (>180 mg/dL). Serums of subjects from the second center will be transported at 4-8°C within 48 hours to the central laboratory. Immediately after the first MMTT, subjects who will choose the intervention group will start with strict gluten-free diet. After the third MMTT at 12 months, subjects and their parents will be allowed to discontinue the diet at their will.

Subjects will be followed up at 3 months intervals. During each visit subjects will undergo a physical examination and their body weight, height and BMI will be measured. Their HbA1c will be measured and their total daily insulin dose noted.

Stool samples will be taken at T1D onset, allocation visit and each 3 months up to 15 months for microbiome analysis and adherence test if the patient belongs to the intervention group. The stool samples will be kept at -80°C until the DNA extraction using the PowerSoil DNA isolation kit (Qiagen, Hilden, Germany). Microbiome profiles will be determined by next-generation sequencing of the V4 variable region of 16S rDNA and processed in a bioinformatic pipeline as described previously [3].

Blood samples for the measurement of regulatory cell subsets (measured by flow cytometry) and their cytokine profiles (after short term PMA-ionomycin re-stimulation) will be taken at baseline and at 6 and 12 months.

Intestinal permeability will be measured through serum zonulin (a marker for dysfunctional gut barrier function) levels at baseline and at 12 months [4]. In addition, urine lactulose/mannitol ratio following the ingestion of lactulose/mannitol solution (mannitol g, lactulose g in 100ml water for injection) and 6 hours urine collection [5] will be performed at 12 months (measured using high-performance liquid chromatography).

End points

Primary

- Area under the curve (AUC) of the C-peptide level during the mixed meal tolerance tests

Secondary

- HbA1c
- Insulin requirement (IU/kg)
- Insulin dose adjusted HbA1c (IDAA1c)
- Gut microbiome composition
- Immune parameters (regulatory cell subsets, cytokine production)
- Intestinal permeability (zonulin concentration and lactulose/mannitol urine excretion ratio)

Data collection nad management

All participants will be assigned a study identification number. The forms with patient data will be stored in a locked room with access only to the team of researchers at both centers. Electronic version of data will be kept in password protected databases in accordance with the GDPR legislature.

Statistical analysis

The analysis of the primary outcome will be based on an ANCOVA model with C-peptide AUC at 12 months as a response and the intervention assignment, baseline C-peptide AUC at visit 1, age, gender and body weight as covariates. The tests and confidence intervals will use robust sandwich variance estimator. The primary analysis will follow the per-protocol principle. Secondly, the results at 6 months will also be analyzed. Analyses of secondary outcomes (HbA1c, insulin dose, immune parameters and gut microbiome composition) will proceed by similar methodology. Longitudinal analyses will be performed by linear generalized estimating equation models with a working independence structure. Linear trends in continuous outcomes

over 12 months will be estimated by the intervention arm and compared with adjustment for sex, age, and baseline body weight SDS.

Ethics and dissemination

The study follows the CONSORT guidlines [6] and was approved by the Ethics Commitee of the 2nd Faculty of Medicine of the Charles University in Prague. The study protocol was registered at ClinicalTrials.gov (NCT 02867436). Written informed consent will be presented and signed by all the participants caregivers. All deviations from the protocol will be monitored and duly noted to assure a safe conduct. The results will be submitted to peer-reviewed journals and abstracts will be presented at national and international conferences. All investigators will have access to the final dataset.

References

1. American Diabetes, A., *(2) Classification and diagnosis of diabetes.* Diabetes Care, 2015. **38 Suppl**: p. S8-S16.
2. Greenbaum, C.J., et al., *Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes.* Diabetes Care, 2008. **31**(10): p. 1966-71.
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4. Esnafoglu, E., et al., *Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects.* J Pediatr, 2017. **188**: p. 240-244.
5. Sequeira, I.R., et al., *Standardising the lactulose mannitol test of gut permeability to minimise error and promote comparability.* PLoS One, 2014. **9**(6): p. e99256.
6. Schulz, K.F., et al., *CONSORT 2010 changes and testing blindness in RCTs.* Lancet, 2010. **375**(9721): p. 1144-6.