Prevalence of ZnT8 Antibody in Turkish Children and Adolescents with New Onset Type 1 Diabetes

Short Running Title: ZnT8 antibody prevalence in Type 1 Diabetes

Selin Elmaogulları1, Seyit Ahmet Uçakturk1, Sehri Elbeg2, Esra Doğer3, Meltem Tayfun1, Fatih Gurbuz1, Aysun Bideci1

1Ankara Children’s Hematology and Oncology Training and Research Hospital, Department of Pediatric Endocrinology, Ankara, Turkey
2Gazi University Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

Address for Correspondence: Selin Elmaogulları MD, Ankara Children’s Hematology and Oncology Training and Research Hospital, Department of Pediatric Endocrinology, Ankara, Turkey
E-mail: ekerbicerselin@yahoo.com
ORCID ID: https://orcid.org/

Conflict of interest: None declared
Received: 11.07.2017
Accepted: 23.09.2017

What is already known on this topic
ZnT8A presence in diabetic children with type 1 diabetes changes according to countries. The prevalence is reported as between %24 and %80.

What this study adds
ZnT8A positivity was 58.2% in Turkish children with type 1 diabetes. ZnT8A was present in 46.6% of cases with negative classic autoantibody scanning.

Abstract
Objective: Zinc transporter 8 protein (ZnT8) is a transmembranic protein organizing the zinc transfer to insulin vesicles. Antibodies formed against ZnT8 (ZnT8ab) are regarded as an independent autoimmunity demonstrator in type 1 diabetes (T1D) diagnosis. Investigation of ZnT8ab prevalence in Turkish children with new onset T1D was planned in this study.

Method: 84 patients between 1-18 years of age diagnosed with T1D between February 2015- March 2016 and the control group consisting of 50 healthy children without any autoimmune diseases were included in the study. Serum samples for Znt8ab level were taken from the patient group in a week after diagnosis the latest and studied with ELISA method.

Results: Znt8ab positivity was detected in 58% of the patients with new onset T1D and 8% of the control group. Znt8ab positivity was shown in 5 out of 11 patients with negative results for other diabetes antibodies (IA-2A, GADA or IAA). No connection was found between Znt8ab positivity and age, gender, presence or degree of ketoacidosis present during presentation, HbA1C, insulin or c-peptide level, thyroid autoantibodies or celiac antibody presence.

Conclusion: Znt8ab prevalence in children with T1D in Turkey was compatible with the data from most countries. The ratio of patients who are clinically considered to have T1D but have negative routine diabetes auto-antibodies were observed to decrease nearly by 50% when ZNT8 was added to the panel. ZnT8 measurement should be more widespread for clarifying the etiology in T1D.

Keywords: ZnT8 antibody, Children, Adolescent, Type 1 Diabetes

Introduction
Type 1 diabetes (T1D) is a chronic disease characterized with immune mediated selective destruction of pancreas beta cells (1). Decrease in the induction effect of infections on negative immuno-regulatory genes due to the genetic predisposition and hygienic way of life becoming more significant in last decades play a role in the start of immune mediated degradation (2). Presence of DRB1*04-DQB1*0302 and DRB1*03 among human leukocyte antigen
A total of 84 patients between 1-18 years of age diagnosed with T1D in Ankara Pediatrics Hematology Oncology Hospital Training and Research Hospital (n=76) and Gazi University Faculty of Medicine (n=8) between February 2015 and March 2016 and 50 healthy children without any autoimmune diseases were included in the study (19). The presence and degree of ketosis or ketoacidosis were recorded at the time of referral (pH 7.3-7.2 low; 7.2-7.1 average; <7.1 severe ketoacidosis). C-peptide level was studied with the serum samples taken during diagnosis using Chemiluminescence Immunoassay method. Patients with C-peptide level above 1 ng/ml were excluded from the study. HbA1C was determined on immune turbidimetry using a modular P800 (Roche Diagnostics, Basel, Switzerland). GAD antibody (GADA) above 1 IU/ml and insulinoma antigen-2 antibody (IA-2A) above 1 U/ml with radioimmunoassay method; insulin antibody (IA) above 0.4 U/ml and anti tissue transglutaminase IgA (tTG IgA) above 18 U/ml with microelisa method were accepted as positivity for these antibodies. Thyroid function tests were evaluated after ketotic or ketoacidotic period and providing euglycemia in the patients. Thyroid stimulant hormone (TSH) and free T4 (fT4) levels were studied with two-regioned and two-staged enzymatic immunoassay methods. According to the reference values of TSH and fT4 kits, TSH lower and upper limit values were accepted as 0.34-5.6 mIU/ml and fT4 lower and upper limits as 0.6-1.2 ng/dl. Anti thyroid peroxidase (anti TPO) and anti thyroglobulin (anti TG) were studied with Beckman Coulter DX1800 chemiluminescence immunoassay method.

ZnT8A was studied with Elisa method on serum samples which were taken within a week after diagnosis and kept at -80C. Medizym anti Zn18 Elisa kit which can detect antibodies developed against Arginin (R-325), Triptofan (W-325) and other non-specific variants was used for this operation with a single measurement and values above 15 U/ml were accepted as positive. ZnT8A presence is shown in nearly 25% of the patients accepted as idiopathic T1D and had negative classic autoantibody scanning (9, 15). Investigation of ZnT8A prevalence in children with new onset T1D and the relation of ZnT8A with other antibodies were planned in this study.

Ethic board consent for the study was taken from the ethic board of Ankara Pediatrics Hematology Oncology Hospital Training and Research Hospital (Consent number: 2015-002).

Statistical Analysis

Statistical analysis of the data was made with “The Statistical Package for the Social Sciences 17.0” (SPSS, Inc. Chicago IL, USA, Microsoft) programme. Values were provided as median±standard deviation. Student t test was used for the comparison of the medians for numeric variables and Chi-square test for comparing the medians for non-numeric variables. Mann Whitney U test was preferred for the evaluation of numeric parameters without a normal distribution. Significance level was accepted as p<0.05.

Results

The mean age of the 84 (49 female, 35 male) cases with T1D was 9.8±4.0 years and 52.4% were prepubertal. In the control group (25 female and 25 male) the mean age was 9.1±4.0 years. It was observed that 22 of the cases referred with (26.2%) hyperglycemia, 23 (27.4%) with ketosis, 39 (46.4%) with ketoacidosis presentation (12 low, 8 average and 19 severe degree of ketoacidosis). Median HbA1C ratio was detected as 11.7±2.3% and C-peptide level as 2.6±0.6 ng/ml.
ZnT8A positivity was detected in 49 (58.2%) cases in T1D patient group and in 4 (8%) individuals in the control group. ZnT8A was present in 5 out of 11(13%)cases with negative GADA, IA-2A and IA. Prevalences of tTG IgA, anti TPO and/or anti TG and diabetes autoantibodies in T1D patients are mentioned in Figure 1. When ZnT8A positive (ZnT8A+) and ZnT8A negative (ZnT8A-) T1D cases were compared, no difference was detected in age, gender, presence and degree of ketoacidosis during referral, Hba1C, insulin or c-peptide level. When they were compared for the prevalence of celiac, thyroid and other diabetes autoantibodies, it was observed that only IA-2A positivity rate was significantly higher in ZnT8A+ cases with T1D (p = 0.024) (Table-1). It was also observed that ZnT8A titers in ZnT8A+ cases in T1D group were significantly high, compared to the ZnT8A+ cases in the control group (Figure 2). While ZnT8A titer was not found related to age and BMI, a positive correlation was detected with C-peptide level (p=0.34). ZnT8A+ cases in the control group were 1, 4.4, 6.3 and 6.9 years old and ZnT8A titers were 93, 49, 45 and 40 U/ml respectively.

Discussion

This study showed that the prevalence of ZnT8A is 58.6% in Turkish children with new onset T1D. This result is in accordance with most of the studies done in other countries. ZnT8A positivity was reported to be between 60-80% in Caucasians (1-18 years old) by Wenzlau et al. (9), 72 in% Czechs (1-19 years old) by Petruzelkova et al. (20) and 65% in Argentinians (10-32 years old) by Faccinetti et al. (21), with new onset T1D. ZnT8A existence was found 24% in Chinese new onset T1D patients (1-70 years old) and difference in HLA genotypes or other inter-ethnic genetic markers were thought to be a possible cause for this low rate (17). However another Asian population, Japanese acute onset T1D patients (19.1±14.5 years old) had 58% ZnT8A positivity as well as Turkish population (22). Araujo et al. from Brazil, whose study contained both Caucasian and non-Caucasian new onset T1D population (30.3 ± 11.4 years old) found an overall ZnT8A existence of 24% and stated that ZnT8A positivity or its titers were not associated with ethnicity (23).

ZnT8A positivity ratio of healthy controls from different countries was reported as 1-2.7% which is a markedly lower ratio than ours (8%) (9, 17, 20, 24). This difference may be attributed to the larger cohorts of those studies which reflect the population better, but still, the possible effect of ethnicity cannot be excluded. In line with the studies using the same analysis method and cut-off value, ZnT8A levels of the control group were lower than the T1D patients (20, 24). ZnT8A was shown to predict progression to T1D risk in first degree relatives of T1D patients (15). Although these healthy controls had negative T1D history in their families, they may have a higher risk for diabetes.

ZnT8A is directed to the epitope at the C terminal of the ZnT8 protein (residues 268-369). The gene polymorphism at the codon for the 325th aminoacid form different variants of ZnT8 proteins and antibodies specific to each of these. These are R325-ZnT8RA, W325-ZnT8WA and rarely Glutamine (Q325)- ZnT8QA variants (9). It was demonstrated that the distribution of antibody variants differed between populations (25). But as triple ZnT8A kit was used in this study, ZnT8A variant distribution in Turkish T1D children could not be determined. Prospective studies following up first-degree relatives of T1D patients or individuals with high risked HLA tissue groups, starting from the first months of their lives until the development of T1D demonstrated that ZnT8A developed many years ago before the development of T1D, in the 9th month the earliest or mostly close to three years of age (9, 26). The youngest ZnT8A+ T1D case in this study was two years old and it may be predicted that the seroconversion started around the age of one when present studies are considered. It was observed that ZnT8A+ prevalence and titer during diagnosis didn't change with age in our study. Anderson et al. detected that ZnT8A prevalence during diagnosis was age-independent in 686 children with T1D (27). In a study examining 227 children and adolescents with T1D, it was shown that ZnT8A prevalence was not related to age at diagnosis but ZnT8A titers increased with age[20]. When the age range of the study is enlarged in the studies, it is observed that both ZnT8A prevalence and titer decreases with latter diagnosis age (16).

In the present study, neither presence nor levels of ZnT8A was found to be related to BMI. Vaziri-Sani F. and Adamo M.’s studies also support this finding (16-28). Differently, Yang et al. reported that ZnT8A positivity was more frequent in leaner than more obese T1D patients but it was mentioned that larger cohorts were necessary to verify this negative association (17).

Whether ZnT8A presence or levels predict residue beta cell function or not is still unclear. Yang et al. showed that the presence and Vaziri-Sani F. et al showed that the presence or levels of ZnT8 were unrelated to c-peptide levels (16, 17). Andersen et al. found that both presence and levels of ZnT8RA and on a lesser level, ZnT8QA were associated with higher levels of stimulated C-peptide after diagnosis and during the follow-up of T1D. But after excluding Znt8RA negative subjects and reanalyzing the relation between ZnT8A levels and stimulated C-peptide
levels, that association failed, which may mean that positivity rather than the level of Znt8RA has a protective role on beta-cell function (29). However, in this present study, it was the opposite; C-peptide levels were positively correlated with the levels, but not the presence of ZnT8A. Different cohort sizes, target ZnT8A epitope and type of C-peptide measurement, fasting or stimulated, may have caused these conflicting results.

Acknowledgments
This work was supported by the Turkish Pediatric Endocrinology and Diabetes Society. References


Figure-1 Frequency of Celiac, thyroid and diabetic autoantibodies in patients with new onset type 1 diabetes (T1D)