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THE ROLE OF ANTI ZINC TRANSPORTER PROTEIN 8 IN CHILDREN WITH TYPE1 DIABETES MELLITUS

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ABSTRACT

Background: Zinc transporter 8 (ZnT8) is a protein highly specific to pancreatic insulin-producing beta cells, it is vital for the biosynthesis and secretion of insulin. ZnT8 autoantibodies (ZnT8A) were detected at the onset of diabetes that lead the researchers to study their role in diagnosis of type 1 diabetes mellitus (T1DM). Therefore, we aim to investigate the serum level of ZnT8A and its association with the pathogenesis of T1DM among pediatric group. **Method:** A case-control study that recruited sixty-eight diabetes patients visiting Pediatric Teaching Hospital, medical city, Baghdad (thirty-four patients diagnosed as T1DM for less than four years regarded as group A and thirty-four patients diagnosed as T1DM for more than four years regarded as group B) and twenty healthy control group that was taken from patients first degree relatives. A sandwich enzyme – linked immunosorbent assay (Elisa) was used to analyse the serum level of ZnT8A which was separated from patients' blood samples. **Results:** The mean age of T1DM patients (N = 68) was 9.43 ± 3.1 years and 10 ± 3 years for 20 healthy controls.34 patients in A group were diagnosed less than 4 years, while the other 34 were diagnosed more than 4 years (group B). Twenty-three of group A (67.6%) tested positive for ZnT8A compared to 5 (14.7%) and 2 (10%) patients tested positive ZnT8A for group B and controls respectively. When ZnT8A-positive and -negative patients within group A and B were compared, no significant differences were detected in relation to gender, age of incidence, time of diagnosis or HbA1C concentration. **Conclusions:** ZnT8A play an important role in the pathogenesis of T1DM and may prove to be a predicting marker for the onset of T1DM.

KEYWORDS: Zinc transporter 8, autoantibody, Type 1, diabetes mellitus, Diagnosis, Child.

INTRODUCTION

The distinctive feature of type 1 diabetes mellitus (T1DM) is the autoimmune damage of pancreatic β cells, involving autoantigens having significant expression and β-cell specificity being targets.[1] It is projected to increase by three percent each year and accounts for 5–10% of all cases of diabetes worldwide[2] Moreover, it is a chronic illness that manifests from the age of five to seven till adolescence, however a diagnosis can be made at any time. In children and teens, Diabetes Mellitus (T1DM) is the most common type, which represent around 90% of DM cases in children aged 15 years old and less.[3] Now, younger teens also get type 2 due to increase obesity among them.[4] In Iraq the prevalence of type 1 DM was 87 per 100,000 with mean age at diagnosis was 15 years old. People who have several first-degree relatives with T1DM are more likely to develop T1DM.[5] Islet-targeting autoantibodies that target insulin, glutamic acid decarboxylase, insulinoma-associated protein 2 and zinc transporter 8, all of which are proteins associated with secretory granules in β-cells, are biomarkers of T1DM-associated autoimmunitythat are found months to years before symptoms onset, and can be used to identify and study individuals who are at risk of developing T1DM.[6] ZnT8, an autoantigen primarily found in pancreatic β-cells, is produced by the SLC30A8 gene and consists of 369 amino acids. This gene is found on chromosome 8's q arm at position 24.11.[7] The zinc required for insulin maturation and/or storage activities in pancreatic beta- cells that secrete insulin is largely provided by ZnT8.[8] As a result, antibodies against ZnT8 may interfere with the production, storage, and secretion of insulin as well as the paracrine/autocrine communication that occurs in islet cells.[9] In addition to heredity, ZnT8 overexpression, SLC30A8 polymorphism, viral infections (including adenovirus, rhinovirus, and Coxsackie), and ZnT8 exposure during insulin release

are all associated with the development of type 1 diabetes mellitus.[10] ZnT8A was detected in about 26% of T1DM patients who tested negative for antibodies against GAD, IA-2, and insulin antigen[11] and In 60% of the Caucasoid population with type 1 diabetics, it has been reported that ZnT8A were discovered. On the other hand, ZnT8A and conventional autoantibodies measured together improved the detection rates to 98%.[12] In children and young adults with T1DM, autoimmunity against pancreatic β-cells is mostly caused by ZNT8, a significant autoantigen. Early in the course of T1DM, elevated levels of ZnT8A are typically found, as the disease progresses ZnT8A titers significantly decrease Researchers have measured and linked ZnT8A to T1DM since ZnT8A is present in patients before the disease develops and typical autoantibodies are absent in T1DM patients[13] ZnT8A has the capacity to add details regarding the prevalence, amounts, and correlation with type 1 diabetes in our community. We therefore set out to determine whether ZnT8A and T1DM are related.

MATERIALS AND METHODS

Sixty-eight diabetes patients who visited the pediatric teaching hospital in Medical City, Baghdad were included in this prospective study. Subjects were categorized into case and control groups. The case group included T1DM patients aged less than 14 years old. Twenty of the first-degree relatives of the patients who matched age and sex were recruited as the healthy control group, and those with diabetes- associated autoimmune disease (i.e. coeliac disease, Hashimoto's thyroiditis) were excluded. Three milliliters of fresh peripheral blood were placed in a gel tube, centrifuged, and the serum immediately frozen in -20°C. Anti-Zinc Transporter 8 was measured using a sandwich (ELISA) kit. 100 µL of the sample was added and bound to antibodies coated on the wells. And then 100 µL of prepared antibody reagent was added and binds to ZNT8 in the sample. Then Streptavidin-HRP was added and bound to the Biotinylated SLC30A8/ZNT8 antibody. After incubation unbound Streptavidin- HRP was washed away during a washing step. Substrate solution then added and color developed in proportion to the amount of human SLC30A8/ZNT8. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm. SPSS ver. 22 was used to analyze the data. The mean +/- standard deviation was used to express all regularly distributed data, whereas the median (interquartile range) was used to express no normally distributed data. Numbers or percentages were used to express categorical data. Whereas, the Fisher exact test or the chi-square test were used to compare frequency differences. The Independent t test was utilized to examine variations in parametric data. Statistics were considered significant if the P value was less than 0.05.

RESULT

In this case-control study, 20 healthy children (12 boys and 8 girls; with mean age for control group was 10 ± 3 years) and 68 T1DM children (36 boys and 32 girls; with mean age 9.43 ± 3.1 years) were involved. We classified 34 patients as belonging to Group A, who had type 1 diabetes mellitus for less than 4 years after diagnosis, and 34 patients as belonging to Group B, who had diabetes for more than 4 years after diagnosis. The demographic characteristics for the patients were shown in table 1.

Table 1: The demographic characteristics for the patients.

Variable	Groups		Total	P value
	A No. (%)	B No. (%)		
Family Hx				
Yes	14 (41.2)	9 (26.5)	23	0.2
No	20 (58.8)	25 (73.5)	45	
Rx				
Injectable	26 (76.5)	32 (94.1)	58	0.04
Pump	8 (23.5)	2 (5.9)	10	
Multivitamins				
Yes	23 (67.6)	22 (64.7)	45	0.79
No	11 (32.4)	12 (35.3)	23	
Sugar Substitutes				
Yes	13 (38.2)	23 (67.6)	36	0.015
No	21 (61.8)	11 (32.4)	32	
Self-Monitoring				
Yes	22 (64.7)	18 (52.9)	40	0.32
No	12 (35.3)	16 (47.1)	28	
Hospitalization				
Yes	27 (79.4)	27 (79.4)	54	0.99
No	7 (20.6)	7 (20.6)	14	
Hypoglycemia				
Yes	18 (52.9)	20 (58.8)	38	0.62
No	16 (47.1)	14 (41.2)	30	

Twenty-three (67.6%) of the patients in group A were positive ZnT8A, compared to just five (14.7%) and two (10%) in the group B and control group, respectively. ZnT8A was found in groups A, B, and C with mean concentrations of 3.074 ± 1.752 ng/ml, 1.912 ± 1.788 ng/ml, and 1.442 ± 0.6 ng/ml, respectively. Figure 1.

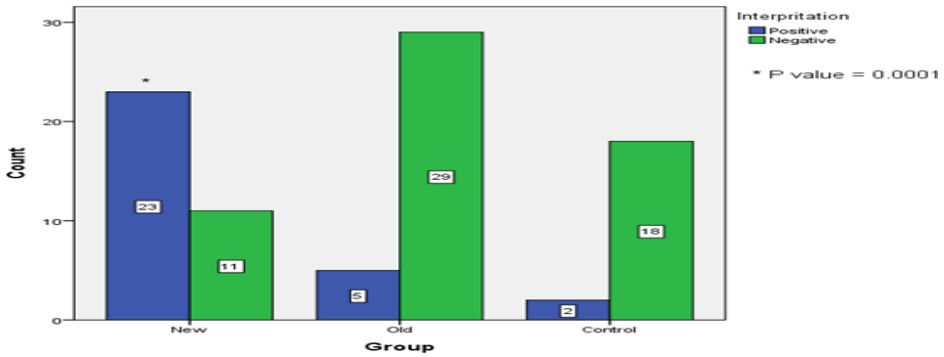

Figure 1. Count of patients with positive ZnT8A in each group.

The concentration of ZnT8A was significantly higher in males in comparison to females of group A (P value = 0.001). There was no statistical difference between male and female of group B and control group (P value = 0.8 and 0.2 respectively). Table 2. ZnT8A-positive and -negative T1DM cases did not differ in terms of glycosylated hemoglobin concentration, age at diagnosis, duration of diabetes.

Table 2: Concentration of ZnT8A comparing between male and female in each group (ng/ml).

Group	Results				P value
	Male		Female		
	Mean	SD	Mean	SD	
A	3.338	1.881	2.630	1.517	0.001
B	1.896	1.402	1.926	2.114	0.8
Control	1.530	726	1.311	.342	0.2

DISCUSSION

Over all, the expression of ZnT8A was detected in 28 DM type 1 patients, which representing 41% of all DM type 1 patients included in our study (N=68). This result was comparable to a results from Latin Americans[14], which demonstrated the ZnT8A in 48% of DM type 1 patients regardless the onset of diagnosis. Also, a large cohort French study demonstrated same results for DM type 1 patients and new onset patients in which ZnT8A expression was detected in 61% of the newly diagnosis patients.[15] However, the importance of ZnT8A is rely on new onset DM type 1. Based on the onset of DM type 1 (group A and group B), the positive ZnT8A result was observed in 23 (67.6%) patients within group A, there were only 5 (14.7%) patients and 2 (10%) individuals showed positive ZnT8A results within group B and control group respectively. This result was highly comparable to Selin's Study from Turkey that showed 58% of new onset DM type 1 have positive ZnT8A and only 8% of control group[16], A review article by Afreen found that expression of ZnT8A have been found in 50 – 60% of DM type 1 patients.[17] The literatures from east Asia (Japan and China) showed relatively lower ZnT8A expression, for Japan it was 36% of patients showed the expression[18] and for China, the expression was observed in 24% of patients, However, Chine's study looks for ZnT8A expression in DM type 1 regardless of the onset of DM type 1, and their main objective was ZnT8A expression relative to other autoantibody markers, for that, their results were lower than what was observed in our study. For Japan's study, the mean age for their patient group was 18 years and this may have a major effect on decreasing the expression of ZnT8A.[19] The mean concentration of ZnT8A was significantly higher in group A in comparison to group B and control group and this observation was similar to Xuan's study. Despite of using ELISA in Xuan's study[20], the prevalence of ZnT8A was lower in their study, while the concentration was in comparison to ours. Italian study on T1DM children, the prevalence of ZnT8A remained substantially stable in patients up to four years from the clinical diagnosis, but then showed a significant decrease in patients ≥5 years from diagnosis.[21] The mean concentration of ZnT8A was significantly higher in males compared to females of group A (P-value = 0.001). There was no statistical difference between males and females of group B and control group A recent study by Bhatty[22] demonstrated no difference in the concentration of ZnT8A between males and females. However, a large study may be needed to establish the role of sex in the concentration of ZnT8A. The mean age in positive ZnT8A of group A, group B and control group was 8 ± 3, 10 ± 3, and 13 ± 1 respectively. There was no significant difference between the mean of each group, which was comparable to the Turkey study that demonstrated no association was found between age and sex with ZnT8A expression. While Japan's study showed that the ZnT8A expression was more frequent in the younger group. Despite the fact, the difference between our study and Japan relies on the difference in the

population of study and ethnicity.[20] For HbA1c, in the positive ZnT8A of group A and group B, the mean was 9.6 ± 1.5 and 10.4 ± 1.5 respectively. There was no significant difference between the mean of each group. The previous study demonstrated the same result regarding HbA1C and it rolled out the correlation between HbA1C and ZnT8A expression. The importance of ZnT8A is relying on its diagnostic features. Inclusion of ZnT8A in the diagnosis panel of DM type 1 will reduce the fraction of patients who lack the autoantibodies (negative) and will enhance the diagnostic sensitivity of up to 90%[23] of new-onset DM type 1 among Iraqi patients. And further study of ZnT8A in correlation with other autoantibodies [namely insulinoma antigen-2 antibody (IA- 2A), glutamic acid decarboxylase antibody (GADA)] could help to establish a near perfect panel for DM type 1 diagnosis.[24, 25]

CONCLUSIONS

The expression of ZnT8A was significantly higher among T1DM patients than controls especially those less than four years' duration of the disease. Also, it was significantly higher in male than female. Clinical applications for ZnT8A in T1DM diagnosis are possible, but more researches are needed. Autoantibodies should be tested for accuracy, sensitivity, and specificity at various cutoff values.

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