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Original Article

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Is Isolated Autoantibody Seropositivity a Risk Factor in the Development of Diabetic Nephropathy in Type 1 Diabetic Children?

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ABSTRACT

Objective: Diabetic nephropathy is one of the most important complications in diabetes mellitus. We aimed to examine the influence of organ-specific antibody seropositivity in diabetic nephropathy.
Methods: Patients with type 1 diabetes and no evidence of celiac disease, thyroid dysfunction, and other kidney diseases and with an annual average HbA1c level <10%, body mass index <25 kg/m^2, and normal blood pressure were enrolled.
Results: Eighty patients (39 boys and 41 girls) were evaluated. Twenty patients with moderately increased albuminuria (diabetic nephropathy group) and 60 patients without albuminuria (control group) showed no statistical difference in age, gender, diabetes duration, age at diagnosis, kidney function tests, and mean blood pressure measurements. Compared to control group, the mean anti-thyroglobulin level and anti-thyroxin peroxidase level were statistically higher in the diabetic nephropathy group, $P = .004$ and $P = .045$, respectively. However, the thyroid function tests were normal in either group.
Conclusion: Determination of the impact of autoantibody seropositivity on the risk of diabetic nephropathy in type 1 diabetic children could be a non-traditional marker in the risk assessment of diabetic nephropathy.
Keywords: Autoimmune diseases, children, nephropathy, Type 1 diabetes mellitus

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INTRODUCTION

Diabetic nephropathy (DN) is one of the most critical complications of type 1 diabetes mellitus (T1DM), which is the most common chronic metabolic disease in childhood.[1] Persistent moderately increased albuminuria (MA) is the earliest sign of DN, followed by a gradual decline in kidney function leading to chronic kidney disease (CKD).[2,3] Microvascular and macrovascular complications, mainly DN, are the leading cause of morbidity and mortality seen in adulthood. Thus, identifying and managing the risk factors should be the primary goal in diabetes therapy.[4] Several risk factors have been determined that affect the onset and progression of DN, such as glycemic control, duration of diabetes, age at onset, puberty, obesity, hypertension, and genetic predisposition.[5] In the management of diabetes, ensuring the optimal weight and the effective control of blood pressure have positive effects on the risk of DN; however, the most important modifiable risk factor in DN is glycemic control.[5-8]

It has long been known that T1DM increases the risk of other autoimmune diseases. Autoimmune processing affecting the pancreas' beta cells may also cause organ-specific and non-organ-specific autoimmune diseases in various tissues and organs along with genetic susceptibility.[9,10] The most frequent concomitant autoimmune diseases of T1DM are autoimmune thyroiditis (AIT), celiac disease (CD), pernicious anemia, vitiligo, and Addison's disease, respectively.[11] In CD, a strict gluten-free diet

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(GFD) may complicate glycemic control due to its high glycemic index and facilitate the risk of DN.[12] Malabsorption and low-grade inflammation in CD also contribute to DN.[12,13] Hashimoto's thyroiditis and Graves' disease are both referred to as autoimmune thyroid diseases (AITD), manifests as hypothyroidism and hyperthyroidism.[14] Hypothyroidism causes hypoglycemia and growth retardation in T1DM, while hyperthyroidism often causes acute diabetic complications such as ketoacidosis and hypertension.[15,16] However, there are some studies that state that long-term hyperthyroidism deteriorates glycemic control as well.[17] It seems that autoimmune disease concomitance contributes to DN by impairing glycemic control with the clinical and laboratory findings they cause. Moreover, most patients with thyroid autoantibodies do not have thyroid dysfunction symptoms.[15] This study was designed to examine the association of organ-specific autoantibody (Ab) positivity without overt clinical symptoms on DN development risk.

METHODS

In this cross-sectional single-center study, T1DM patients followed between January 2016 and January 2019 in pediatric endocrinology outpatient clinics were retrospectively evaluated. Among these patients, those who met the following criteria were enrolled in the study; Patients diagnosed with T1DM according to the American Diabetes Association criteria;[18] age at diagnosis <18 years; disease duration <10 years; annual average HbA1c level <10%; body mass index (BMI) <25 kg/m^2; and normal outpatient blood pressure measurements. Patients with missing data were excluded. Eighty patients who met the inclusion criteria were divided into 2 groups: DN group and without persistent MA (control group). These 2 adjusted groups were compared in terms of organ-specific Ab levels (anti-thyroglobulin (anti-Tg), anti-thyroxin peroxidase (anti-TPO), and anti-tissue transglutaminase IgA (anti-tTG). Patients with Ab measurement above the defined upper limit without any overt clinical symptoms were considered seropositive.

Patients with positive anti-tTG IgA were referred to the pediatric gastroenterology outpatient clinic. Those with signs and symptoms compatible with CD and positive anti-endomysial (EMA) IgA results were further evaluated for CD. However, they were excluded from the study. Patients who were anti-tTG IgA positive, anti-EMA IgA negative, and had no apparent signs of CD were included in the study. However, diagnostic endoscopy was not performed in this group.

Patients with positive thyroid-specific Abs (anti-Tg and/or anti-TPO) were further evaluated in the pediatric endocrinology outpatient clinic. Those with signs and symptoms compatible with hypothyroidism or hyperthyroidism, abnormal thyroid function tests, and abnormal thyroid ultrasonography results were excluded from the study. Patients who were thyroid-specific Ab seropositive with normal thyroid function tests and had no overt signs of thyroid dysfunction, whether they had normal or abnormal thyroid ultrasound findings, were included in the study.

Patients with persistent MA were further evaluated in the pediatric nephrology outpatient clinic. Those with suspected accompanying kidney disease in anamnesis, physical examination, laboratory, and radiological evaluation were excluded from the study.

Diagnosis of DN was made clinically; a diagnostic kidney biopsy was not planned. These patients were followed up in 3 months periods by 24-hour urine protein excretion, kidney function tests, and blood pressure measurements. In patients with high blood pressure, increasing protein excretion in the follow-up period angiotensin-converting enzyme (ACE) inhibitors were started at an appropriate dose.

The Abbott i2000 hormone analyzer using the luminescent immunoassay method evaluated the thyroid-specific abs, anti-TPO, and anti-Tg. Accepted normal serum levels of thyroid function tests were as follows: TSH 0.5-4.3 mU/L, free thyroxine (fT4) 0.93-1.70 ng/dL, and accepted normal serum levels of thyroid-specific Abs. Anti-tTG IgA Ab levels were as follows: anti-TPO 0-34 IU/mL, anti-Tg 0-34 IU/mL, and anti-tTG <30 U/L.[19]

HbA1c was evaluated with the HPLC method. Body mass index was calculated as the ratio of body weight (kg) to squared height (meters). Blood pressure was evaluated according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents.[20]

All patients in this study were evaluated with 24-hour urine collection. Persistent moderately increased albuminuria was defined as an albumin excretion rate of 30-300 mg/24 hour on 2 or more 24-hour urine sample results within 3-6 months.[21]

The study was performed following the Helsinki Declaration, and all patients/parents have given their informed consent to participate in the study. The study was approved by the Local Ethics Committee (11/12/2020-13-27).

MAIN POINTS

- This study shows that organ-specific autoantibody seropositivity without any clinical and laboratory overt symptoms may be associated with the development of diabetic nephropathy along with poor metabolic status in diabetic children.
- Isolated autoantibody seropositivity may be used in the risk assessment of diabetic nephropathy, alongside the identified traditional risk factors in the future.
- It is suggested to keep the anti-thyroid autoantibody positivity cutoff value lower in diabetic patients.

Statistical Analysis

All data were analyzed using the Statistical Packages for Social Sciences version 21.0 software (IBM Corp.; Armonk, NY, USA). The chi-squared test was used to analyze categorical variables. The distribution of the variables was tested by the 1-sample Kolmogorov–Smirnov test. Non-parametric data were presented as the median and compared by the Mann–Whitney *U* test and the Kruskal–Wallis test where appropriate. Parametric data were presented as mean (± SD) and compared by Student's *t*-test and analysis of variance where appropriate. The relationship between moderately increased albuminuria level and other variables were analyzed by linear regression analysis. The relationship between DN and ab seropositivity was analyzed by binary regression analysis. The correlations between variables were evaluated by Spearman's correlation test. A *P*-value of <.05 was considered statistically significant.

RESULTS

In this study, 80 patients who met the inclusion criteria were evaluated. There were 39 boys (48.75%) and 41 girls (51.25%). The mean age and age at diagnosis of diabetes of the patients were 14.2 ± 3 (range 7-18) years and 7.21 ± 3 (range 0.6-12.3) years, respectively. The mean diabetes duration was 7.07 ± 1.25 (range 5-10) years. The mean BMI was 18.9 ± 2.4 (range 15-24) kg/m^2. The mean estimated glomerular filtration rate (GFR) was 117.25 ± 31.8 mL/min/1.73m^2 (range 61.7-148.5). The mean annual HbA1c value was 8.7 ± 0.8% (range 7-10).

Persistent MA was detected in 20 of 80 patients (25%). As the groups were matched, 20 patients with persistent MA (DN group) and 60 patients without persistent MA (non-DN group) showed no statistical difference in age, gender, diabetes duration, diagnosing age, BMI, HbA1c, and, estimated GFR values. The mean MA level was 116.3 ± 105.6 (median 74.5, range 25-300) mg/24h in the DN group, and 7.7 ± 5.4 (median 5.5, range 1-20) mg/24h in the non-DN group (*P* = .001).

A statistically significant difference was observed when DN and non-DN groups were compared in terms of anti-Tg and anti-TPO levels. The mean anti-Tg Ab level of DN and non-DN groups were 21.83 ± 58.13 (range 0.25-244) IU/mL and 5.80 ± 29.63 (range 0.04-131) IU/mL, respectively. The mean anti-Tg level was significantly higher in the DN group (*P* = .004). The mean anti-TPO level of DN and non-DN groups were 153.16 ± 385.85 (range 0.1-1609) IU/mL and 18.17 ± 128.96 (range 0.01-800) IU/mL, respectively. Similarly, the anti-TPO level was significantly higher in the DN group (*P* = .045). However, there was no clinical or laboratory evidence of thyroid dysfunction in either group. The mean anti-tTG IgA level of DN and non-DN groups were 26.56 ± 61.18 (range 0.21-136) U/L and 5.80 ± 14.42 (range 0.01-79) U/L, respectively. There was no statistically significant difference between groups in terms of anti-tTG IgA ab levels (*P* = .055). The demographic and laboratory characteristics of both groups were summarized in Table 1.

Table 1. The Clinical and Laboratory Characteristics of Both Groups

Groups	DN (n = 20)	Non-DN (n = 60)	*P*
Male	6 (30%)	33 (55%)	.49
Female	14 (70%)	27 (45%)	
Age (years)	15.5 ± 2.5	14.16 ± 3.1	.38
eGFR (mL/min/1.73 m^2)	115 ± 27.4	118 ± 34.5	.36
BMI (kg/m^2)	19 ± 1.93	19 ± 2.6	.63
Duration of DM (year)	6.54 ± 0.9	7.04 ± 1.3	.11
HbA1c (%)	8.91 ± 0.7	8.6 ± 0.8	.29
Anti-Tg (U/mL)	21.83 ± 58.13	5.80 ± 29.63	**.004**
Anti-TPO (U/mL)	153.16 ± 385.83	18.17 ± 128.96	**.045**
Anti-tTG IgA (U/L)	26.56 ± 61.18	5.80 ± 14.42	.055
MA (mg/24 h)	116.3 ± 105.6	7.7 ± 5.4	**.000**
TSH (mU/L)	1.75 ± 1.1	1.6 ± 0.8	.24
sT4 (ng/dL)	0.97 ± 0.7	0.86 ± 0.8	.53
Anti-thyroid Ab positivity (%)*	45% (9/20)	11.6% (7/60)	**.001**
Anti-celiac Ab positivity (%)**	20% (4/20)	18.3% (11/60)	.86
Double Ab positivity (%)***	5% (1/20)	3.5% (2/60)	

*Anti-Tg and/or anti-TPO seropositivity (Ab titer > 34 U/mL).
**Anti-tTG IgA seropositivity (Ab titer > 34 U/L).
***Anti-Tg and/or anti-TPO and anti-tTG IgA seropositivity.
BMI, body mass index; Ab, autoantibody; DM, diabetes mellitus; DN, diabetic nephropathy; MA, moderately increased albuminuria; TPO, thyroxin peroxidase; TSH, thyroid-stimulating hormone; tTG, tissue transglutaminase.
P < .05 is statistically significant.

Spearman's correlation analysis showed no statistically significant correlation between DN and current age, gender, age at diagnosis, diabetes duration, BMI, and mean HbA1c levels. The anti-TPO and anti-Tg Ab levels had a moderate positive correlation with MA levels (Spearman's rho = 0.42, *P* = .015 and Spearman's rho = 0.46, *P* = .008, respectively). Also, there was a weak but statistically significant correlation between the mean HbA1c and diabetes duration (Spearman's rho = 0.225, *P* = .011). In linear regression analysis, there was a statistically significant correlation between MA level and anti-Tg Ab level (*P* = .001 odds ratio (OR): 0.842, 95% CI: 0.357-1.327).

A statistically significant difference was observed when DN and non-DN groups were compared in terms of anti-thyroid ab seropositivity (*P* = .001); however, a similar relationship was not shown in anti-CD A seropositivity (*P* = .86). In addition, there was a statistically significant correlation between DN and anti-thyroid Ab seropositivity (phi = 0.361, *P* = .001). Binary logistic regression analysis showed a statistically significant correlation between DN and anti-thyroid Ab seropositivity as well (*P* = .002, OR: 0.156, 95% CI: 0.047-0.517). The detailed comparison of thyroid function tests,

Ab levels, and Ab seropositivity of groups was summarized in Table 1.

DISCUSSION

Clinically overt microvascular complications, particularly advanced DN, are rarely seen in childhood. However, early structural and functional subclinical abnormalities can be detected even a few years after a diabetes diagnosis.[22] Persistent MA is the most common finding during this phase that progresses to CKD in adulthood.[23] There are many genetic and metabolic risk factors and individual differences, and there may be other as yet undefined factors that determine this process starting with MA to CKD.[6,7] Since urinary protein excretion increases during the daytime with prolonged duration in the upright position, particularly in the 3-5% of the adolescent population, it is important to get urine samples from the first urine in the morning to rule out orthostatic proteinuria.

Identifying and managing as many risk factors as possible in diabetic patients is mandatory in risk assessment in the process of leading DN. Several risk factors have been determined that influence the onset and progression of DN, such as duration of diabetes, age at onset, puberty, and family history of diabetic complications which are non-modifiable factors. Also, glycemic control, obesity, and high blood pressure are some of the modifiable risk factors that have been shown in many studies. The most critical risk factor is glycemic control which is under the influence of many modifiable and non-modifiable risk factors such as puberty and obesity.[5,6-23] As is known, HbA1c has long been used for monitoring glycemic control. However, there is not a clearly defined specific HbA1c threshold, suggesting that DN will not emerge. Although it remains a little controversial, it is recommended to keep the HbA1c level below 7% in the management of diabetic patients.[24] In our study. the mean HbA1c level was 8.7 ± 0.8%, although this value was above the recommended level, the mean HbA1c levels were matched in both groups as the mean HbA1c levels were 8.91 ± 0.73 and 8.64 ± 0.85 respectively (P = .29).

The duration of T1DM is another closely related risk factor of DN. In a study by Amin et al.[7] the cumulative prevalence of MA was 26% after 10 years of duration and 51% after 19 years of duration diabetes. In a study by Salgado et al.[25] DN was seen in 20.9% of patients with a mean follow-up duration of 11.32 ± 4.02 years. In our study, DN was detected 20 of 80 patients (25%) with a mean follow-up duration of 7.07 ± 1.25 years. Poor glycemic control of the patients in our study is one of the factors that may explain the earlier development of DN.

On the other hand, in our study, the anti-thyroid Ab (anti-Tg and anti-TPO) positivity rate was 20% (16/80 patients). In a study by Monteagudo et al.[26] thyroid Ab positivity without thyroid dysfunction was 17.6%. Similarly, in a study by Hansen et al.[27] thyroid Ab positivity was 16% in diabetic patients with normal thyroid function tests. Since thyroid autoantibodies in T1DM patients increase with age and diabetes duration, some studies have a higher incidence of thyroid Ab positivity.[15] However, in our study, a significantly higher thyroid Ab positivity was shown than in studies in which patients with similar age and diabetes duration were evaluated. Thus we speculate that the high rate of MA in our study also supports the possible relationship with thyroid Ab seropositivity even without any thyroid dysfunction signs and symptoms. It is difficult to explain the higher Ab seropositivity in our study than in similar studies; however, this may be an autoimmune genetic predisposition in a region where consanguineous marriages are common.

Celiac disease is another concomitant autoimmune disorder seen in T1DM, with an incidence of 3%-16%.[28] The diagnosis of CD is based on specific clinical manifestations, 10-fold increased anti-tTG Ab positivity and positive anti-EMA, however in T1DM majority of patients may not present with classic CD symptoms, therefore in these patients, small intestine biopsy confirmation is needed.[29] Studies evaluating the impact of CD on DN have conflicting results. Rohrer et al[22] revealed that CD is an independent risk factor for DN in T1DM patients, in a study of adjusted groups for age, sex, duration of diabetes, blood pressure measurements, and mean HbA1c levels. Studies are reporting an increased rate of microvascular complications and others report no difference. However, these studies have considered different modifiable and non-modifiable risk factors.[30,31] Studies reporting that CD has an impact on DN generally focus on the following mechanisms: malabsorption, the high glycemic index of GFD, and inflammation which are related to the overt CD.[12,13] Patients that are seropositive with no gastro-intestinal or extra-intestinal manifestation of CD are defined as a silent CD.[31] Therefore, we can expect no increased risk in DN since the mentioned mechanisms do not exist in these patients. Similarly, in the study by Leeds et al.[30] it is reported that seropositivity of anti-tTG antibody alone has an insufficient effect on noticeable microvascular complications, and enteropathy is required in T1DM. It has long been known that there is a relationship between CD and neuropsychiatric complications, which can also occur in patients with positive celiac serology in the absence of enteropathy.[33] Although the exact mechanisms are not fully understood, this situation may be due to the presence of neural antigens that cross-reacting with gliadin.[34] Similarly, it is possible that anti-thyroid Abs may interact with kidney antigens to increase the development of DN. In a study by Hoffmann et al.[13] two groups with DM and DM with AIT were compared in terms of skin microcirculation. In this study, both groups were matched for risk factors and had normal thyroid function (TSH, sT4) tests. Interestingly, it was observed that microcirculation has deteriorated in DM with the AIT group. Hence, another speculation of our results could be the stated dynamics that alter skin microcirculation may contribute to nephropathy in diabetic patients by affecting kidney microcirculation. When we interpret our results from another

perspective, the question arises whether anti-thyroid Ab seropositivity is the cause or the result of poor glycemic control in DN. The fact that the study groups were matched in terms of the mean HbA1c levels and the significantly higher anti-thyroid ab seropositivity in the DN group suggests that Ab seropositivity is also an effective factor in DN. However, the actual reason for Ab seropositivity is still unclear, genetic predisposition may be one of the reasons.

Since in our study the mean anti-Tg and anti-TPO Ab levels in the DN group were significantly higher than in the control group, earlier and closer MA follow-up may be recommended with diabetic children at relatively high anti-thyroid Ab levels, even within the normal range. In addition, keeping the Ab seropositivity threshold low in diabetic children may be another recommendation.

CONCLUSION

In conclusion, determining the association between ab seropositivity and DN in children can be a crucial non-traditional marker in the risk assessment. However, to ascertain the association of ab seropositivity with DN and to clarify the mechanisms of these interactions, further studies with larger antibody panels and sample sizes are needed.

Ethics Committee Approval: This study was approved by Ethics Committee of Mustafa Kemal University (Approval No: 13-27, Date: 11/12/2020).

Informed Consent: Verbal informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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