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Association between Parvovirus B19 and thyroid/celiac autoantibodies among T1DM pediatric patients

H.C. CHANDRAMOORTHY[1,2], A.A. SHATI[3], A.A. ASEERI[3], A. SUBRAMANIAN[1], A. ALHANSHANI[3], E.A.H. ALMOHSENY[1,3], R. RAM MANI[4], A. KUMAR[1], R.A. ALI MOOSA[1], A. AL-HAKAMI[1,2], K. CHIDAMBARAM[5]

[1]Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, Abha, Saudi Arabia
[2]Center for Stem Cell Research, College of Medicine, King Khalid University, Abha, Saudi Arabia
[3]Department of Child Health, College of Medicine, King Khalid University, Abha, Saudi Arabia
[4]Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur, Malaysia
[5]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia

Abstract. – OBJECTIVE: In recent years, an overwhelming association between Pediatric Type 1 Diabetes Mellitus (T1DM) and autoimmune diseases has been largely reported. The current study was designed to determine a possible association between autoimmune thyroiditis (AIT), celiac disease (CD) - associated autoantibodies, and Parvovirus B19 infection among pediatric T1DM cases in the southwestern region of Saudi Arabia.

PATIENTS AND METHODS: Blood samples from age groups 1-18 years attending the Diabetic Clinic were collected over a period of 12 months. Serum anti-thyroid peroxidase (TPO), anti-thyroglobulin (TG), anti-tissue transglutaminase immunoglobulin A (TG-IgA), endomysial IgA (EMA-IgA), Parvovirus B19-IgG and IgM antibodies were detected by standard methods.

RESULTS: The results showed the prevalence of autoantibodies against thyroid and CD among pediatric T1DM patients to be 44 (25%) and 25 (14.4%), respectively. The prevalence of antibodies against B19 was 70 (40%). Further determination of the prevalence of Parvovirus B19-IgG antibodies and thyroid antibodies among T1DM pediatric patients revealed that there was a significant association between them with a $p<0.0491$.

CONCLUSIONS: The prevalence of autoantibodies against the thyroid was higher among the seropositive Parvovirus B19 children with T1DM. A positive association between the prevalence of autoantibodies against thyroid disease and the increase in the duration of diabetes was also noted. Hence, periodic screening of T1DM patients for B19 antibodies and autoantibodies for thyroid is crucial.

Key Words:
Type I Diabetes Mellitus, B19 Parvovirus, Celiac autoantibodies, Thyroid autoantibodies, Autoimmune thyroiditis.

Introduction

Type 1 diabetes mellitus (T1DM) is a well-known autoimmune disease commonly found in children and adolescents[1]. The global estimation of annual pediatric T1DM incidence growth rate is 3-5%[2], and Saudi Arabia has marked the fourth nation in the world[3]. Many autoimmune diseases like celiac disease (CD), autoimmune thyroid disease (AIT), Addison's disease (AD), and vitiligo disease are associated with T1DM and are more often concomitant with AIT and CD[4,5]. Thus, T1DM patients possess a greater risk of developing AIT[6]. Some studies[5,7,8] revealed that thyroid autoimmunity was two to three times more commonly found in patients with T1DM than in healthy individuals. Among the T1DM patients, 50-80% have thyroid antibodies (TAb), and nearly 50% are known to develop clinical AIT[9-11]. When compared with a healthy population, the prevalence of 0.5% CD among T1DM patients is 4.4-11.1%. Studies[12,13] suggested a rise in the incidence of celiac autoantibodies among T1DM children, in which the celiac antibodies prevalence ranged between 3.9-16.8%. An increase in the frequency of thyroid autoantibodies and CD-related antibodies among T1DM patients and regular assessment of TAb and CD-related antibodies is crucial for the timely detection of autoimmune thyroid disorders and overt CD[5,14].

Some studies[15,16] indicated that viral infections are a significant factor associated with thyroid disorders. Viruses radiate the most commonly observed environmental factors that induce autoimmunity in T1DM patients[17,18]. A wide spectrum of

Corresponding Author: Harish C. Chandramorthy, MD; e-mail: hshkonda@kku.edu.sa

autoimmune diseases in association with Parvovirus B19 has been explored in recent years. However, scanty studies[19-22] have pictured the development of autoimmune thyroiditis and celiac disease with B19 virus infection. Moreover, no substantial data are available regarding the prevalence and association of thyroid autoantibodies, CD-related antibodies, and parvovirus B19 antibodies among T1DM pediatric patients. In the present study, the association and frequency of autoimmune markers like thyroid autoantibody, CD-related antibodies, and Parvovirus B19 antibodies among pediatric patients with TIDM have been studied.

Patients and Methods

Study Design

This descriptive cross-sectional study was conducted over a period of 12 months. The sample population was composed of patients 1-18 years old diagnosed with T1DM who regularly visited the Aseer Diabetes Center. The study was approved by the Research Ethics Committee at the College of Medicine, King Khalid University (REC#2015-04-04). An informed consent was obtained from the parents/guardian or patients following the relevant national regulations and institutional policies. The diagnosis of T1DM was confirmed to follow the standard American Diabetes Association guidelines. The criteria were fasting plasma glucose to be ≥ 126 mg/dL (7.0 mmol/L) while 2-hour plasma glucose levels to be ≥ 200 mg/dL (11.1 mmol/L) through an oral glucose tolerance test or through hemoglobin A1C (HBA1C) to be ≥ 6.5% (48 mmol/mol) and/or randomly done plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.

Sample Size

A total of 174 TIDM-diagnosed children/adolescents and individuals reaching 18 years of age still followed by a pediatrician for practical purposes were included in the current study. Briefly, 5 ml of venous blood sample was collected from each patient after obtaining their assent. Blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged for 10 minutes at 1,000 rpm. Aliquots of serum samples were stored at -80°C. The samples were tested for autoantibodies against the thyroid, CD-associated autoantibodies, and Parvovirus B19 antibodies.

Detection of Antibodies Against Thyroid, Parvovirus and Celiac Disease

The aliquots of serum were thawed and tested. Serum anti-thyroid peroxidase (TPO), anti-thyroglobulin (TG), anti-tissue transglutaminase IgA (TG-IgA), endomysial IgA (EMA-IgA), Parvovirus B19-IgG and -IgM antibodies were detected using standard kits by standard methods.

Anti-Thyroid Peroxidase (TPO), Anti-Thyroglobulin (TG)

TPO-antibodies (Ab) and TG-Ab were detected by indirect Enzyme-Linked Immunosorbent Assay (ELISA) using commercial kits from Human Diagnostics (Wiesbaden, Germany). The upper normal limit for anti-Tg antibodies was set at 200 IU/mL. However, the upper normal limit for the anti-TPO antibodies was set at 150 IU/mL.

CD-Associated Autoantibodies

For CD-associated autoantibodies, anti-EMA-IgA was detected using primate smooth muscle slides using indirect Immunofluorescence (Immco Diagnostics, Buffalo, USA). Two independent observers examined the slides for the authenticity of the results. Anti-tissue transglutaminase IgA was detected by indirect immunofluorescence (Immco Diagnostics, Buffalo, USA).

Parvovirus B19 Antibodies

Parvovirus B19-IgG and IgM-specific IgG antibodies were quantified by indirect ELISA using commercially available kits (Virion/Serion, Wurzburg, Germany). Results were calculated based on the following formula:

For IgG

Upper cut-off value = Mean standard OD*0.466.
Lower cut-off value = Mean standard OD*0.301.

For IgM

Upper cut-off value = Mean standard OD*0.523.
Lower cut-off value = Mean standard OD*0.359.

Patients with an OD < upper cut-off value were considered positive, and those with OD > lower cut-off value were considered negative.

Statistical Analysis

Definite variables were presented in 'n' (%), whereas continuous variables were presented with median or mean±SD. Univariate analysis testing was performed using the Fisher exact test for definite variables with $p<0.5/p<0.01$, which was considered to be statistically significant. The

analysis was done using GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA).

Results

Out of the 174 patients evaluated, 93 (53.4%) were females. The majority of samples were from the adolescent age group of 10-18 years, while few were from the age group between 5 and 10 years. The youngest age of presentation was 1 year and 6 months. The median age of the patients was 11 years and 5 months, with a 95% confidence interval (CI) ranging from 10.8 to 12.2. The mean age at diagnosis was 6.8 years (SD=3.6) (Figure 1).

The prevalence of autoantibodies against thyroid was 44 (25%) and celiac disease 25 (14.4%) among T1DM patients (Figure 2). The prevalence of antibodies against Parvovirus B19 was 70 (40%). Patients with positive autoantibodies against the thyroid are 8% and 5% for TPO and TG, respectively. However, both antibodies were present in 12% of the total patients (Figure 3). The prevalence of Parvovirus B19 IgG antibodies and thyroid antibodies among T1DM pediatric patients revealed a significant association between them (Table I). Furthermore, the duration of TIDM and the positivity of thyroid antibodies were significant (p=0.009) (Table II). The prevalence of various CD-associated autoantibodies was determined (Table III), and a positivity of 10.9% for both EMA-IgA and tTG-IgA was obtained.

Discussion

The current results report the prevalence of autoantibodies against thyroid and celiac disease

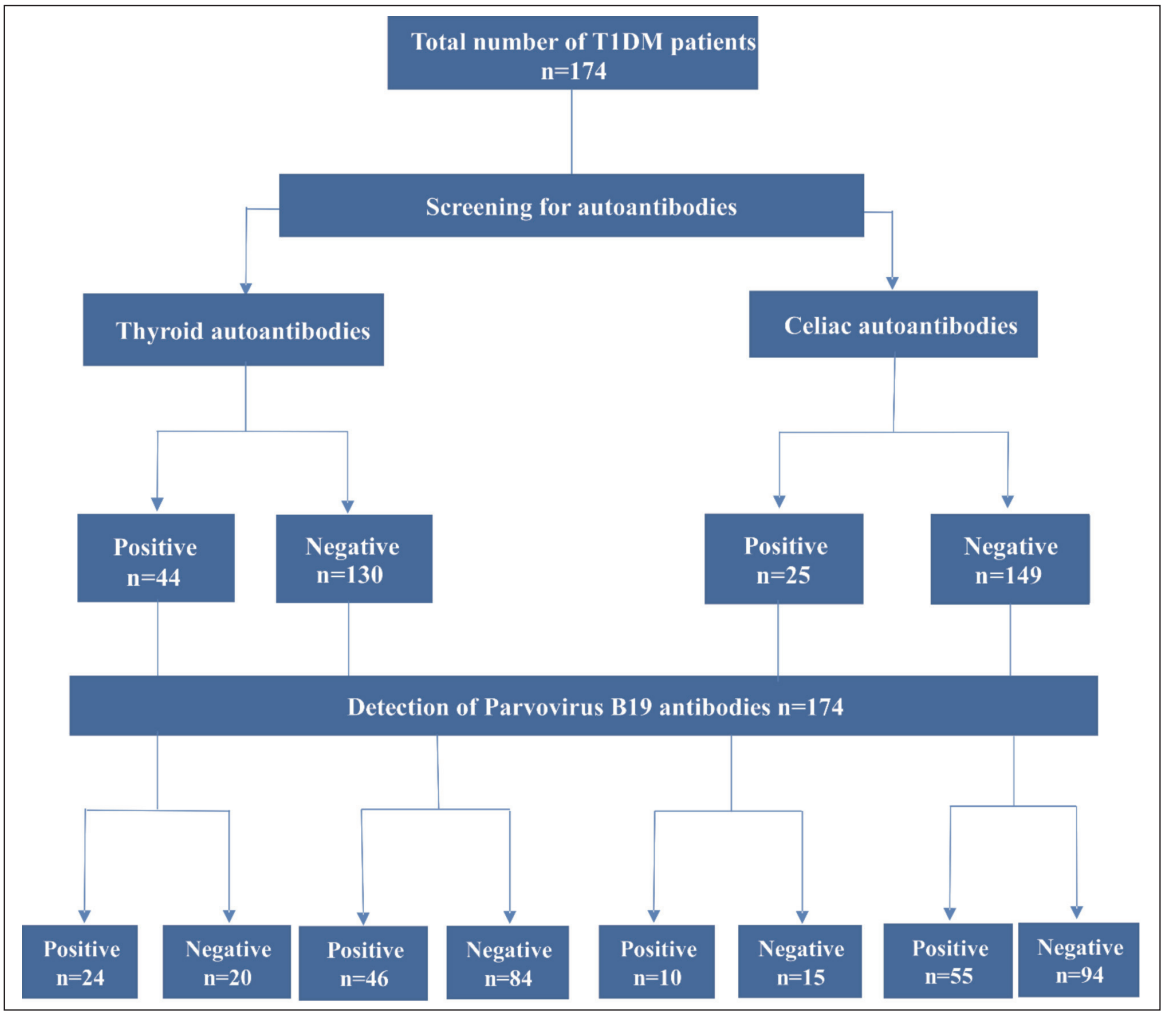

Figure 1. Flow chart. Prevalence of autoantibodies against thyroid and celiac disease-associated autoantibodies in T1DM patients and the seropositivity of Parvovirus B19 among them.

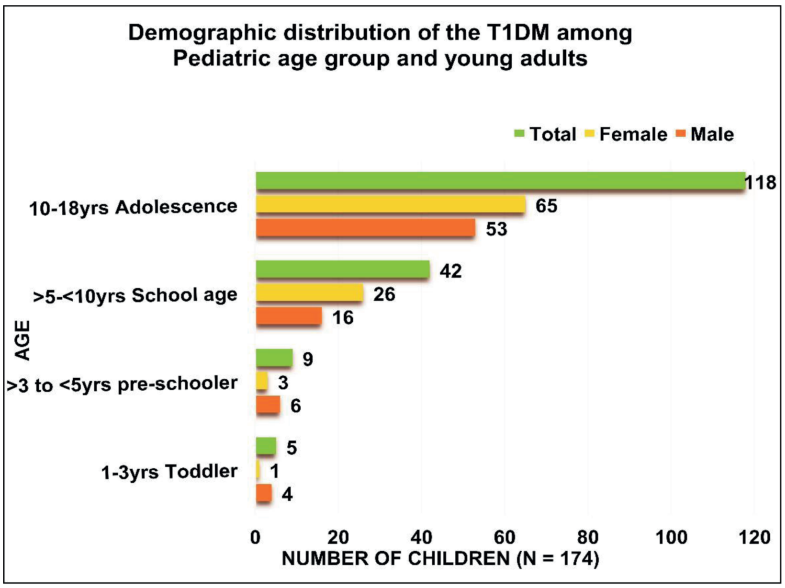

Figure 2. Demographic distribution of T1DM among the pediatric age group.

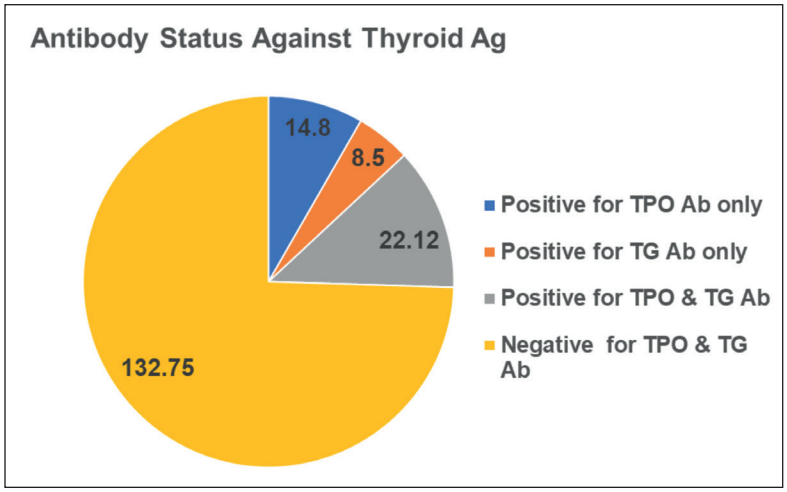

Figure 3. Figurative data of autoantibodies against thyroid among T1DM pediatric and young children. TPO: Anti-thyroid peroxidase, TG: Anti-thyroglobulin, Ab: antibodies.

Table I. Association between the presence of thyroid autoantibodies and Parvovirus B19 antibodies among T1DM patients.

Thyroid autoantibodies among T1DM	Parvovirus B19 antibodies (positive)			
	IgG		IgM	
	Positive	Negative	Positive	Negative
Positive	23 (52.2%)	21 (47.7%)	2 (4.5%)	42
Negative	45 (34.6%)	85 (65.3%)	3	127
Total	68	106	5	169
p	0.0491		0.6015	

This result is significant with a $p<0.05$.

and evaluate their association with Parvovirus B19 seropositivity among children and teenagers with T1DM. We established a significant association between the presence of thyroid autoantibodies and their seropositivity to Parvovirus B19 ($p=0.04$). The outcomes show an increase in the level of thyroid

Table II. Association between duration of T1DM and the positivity of thyroid antibodies.

Thyroid antibodies	Duration of T1DM		
	≤3 yrs	>3 yrs	Total
Positive	12	32	44
Negative	65	65	130
Total	77	97	174
p		0.009	

The Fisher exact test statistic value is 0.009.

Table III. Descriptive statistics of CD- associated autoantibodies among T1DM pediatric patients.

Total no. of participants (n=174) Markers detected	Celiac disease associated autoantibodies status (%)
Positive for EMA-IgA only	5 (2.9)
Positive for tTG-IgA only	1 (0.6)
Positive for EMA-IgA and tTG-IgA	19 (10.9%)
Negative for EMA-IgA and tTG-IgA	149 (85.6%)

CD = celiac disease; EMA = antibody to endomysium, tTG = antibody to tissue transglutaminase; GA = immunoglobulin A.

autoantibodies among B19 seropositive T1DM children, which needs to be explored in future studies. However, no significant association was found between B19 seropositivity and antibodies against celiac disease, indicating that regular screening of patients with T1DM is crucial to identify Parvovirus B19 antibodies and thyroid autoantibodies.

In the current study, the thyroid antibody prevalence was found to be 25%. Similar reports[1,23] have presented a range of 12-23.4% among young patients. Among the 25% of patients with anti-thyroid antibodies, 23 (52%) had anti-TPO, and 16 (36%) had anti-TG. The prevalence of anti-TPO among T1DM ranges between 25% and 31% in other studies[9,24]. A study by Umpierrez et al[25] suggested that the prediction of hypothyroidism based on anti-TPO was found to have a 67% positive and 90% negative predictive value, indicating a robust association between the presence of thyroid-auto antibodies and possible thyroid dysfunction in the future among children with T1DM[26,27].

In the current observation, 93 (53.4%) of the samples were from female cases. The mean age at diagnosis was 6.8 years (SD=3.6). The median age of the patients was 11 years and 5 months, with a 95% confidence interval ranging from 10.8 to 12.2. The results were consistent with the results obtained by Habeb et al[28] in a study focusing on children in Saudi Arabia, where a greater incidence was observed among children aged 10-12 years compared to younger kids. Other studies[29,30] have documented that the peak incidence correlates with puberty at (10 to 14 years of age). Moreover, the varied prevalence of thyroid autoantibodies based on geographic and cultural diversities has been observed[31] against individuals with T1DM. The mean duration of diabetes was 5.1 (95% CI: 4.36-5.84). Furthermore, the results showed that there was a significant correlation between the prevalence of antithyroid antibodies and diabetes duration. The prevalence was higher among patients with diabetes for > 3 years (p= -0.0009). A similar association has been observed in a study by Kakleas et al[1].

The autoantibodies among T1DM should be against CD. Thus, the International Society of Pediatric and Adolescent Diabetes recommends screening of CD every 1-2 years during the diagnosis of diabetes[32,33]. Hence, we determined its prevalence, and it was found to be 14.4%, consistent with other studies[12,13,33] with a prevalence range of 9.87-16.8%.

Limitations

The major limitation was the limited sample size; moreover, in many cases, we could not get follow-up of the cases. The study results warrant a larger cohort spanning several months across seasons to know the real burden of the Parvovirus B19 association with T1DM as well as the development of thyroid autoantibodies in these cases. It may be noted that the prevalence

of thyroid antibodies along with the Parvovirus B19 infection, which is a predominantly pediatric infection, needs to be assessed to know the exact association between them as well as their trilateral relationship with T1DM.

Conclusions

This study observed an increase in the prevalence of thyroid autoantibodies against the seropositive Parvovirus B19 children with T1DM. A positive association was found with an increase in the autoantibody's prevalence against thyroid disease and when diabetes was advanced.

Hence, regular screening of T1DM patients is crucial to identify B19 antibodies and thyroid autoantibodies. Further studies are required to establish the time of positivity of B19 antibodies detection and the development of thyroid antibodies among T1DM children.

Conflict of Interest
All authors declare no conflict of interest.

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Ethics Approval
The study was approved by the Research Ethics Committee at the College of Medicine, King Khalid University (REC#2015-04-04), and informed consent was obtained from the patients' parents following the relevant national regulations and institutional policies.

Availability of Data and Materials
Material and data that support the findings of this study are available upon request to the corresponding author.

Authors' Contributions
HCC, AS, and AAH conceived the idea and wrote the initial manuscript. AH, AAA, and AAS collected the samples. EAHA, RAAM, and HCC processed the samples. AK, AS, and HCC analyzed the results. HCC, RRM, KC, and AAS validated the results. HCC, AAS, and AAA corrected the paper. AS, RRM, KC, and AHH reviewed the final draft. All the authors approved the submission.

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Informed Consent
Informed consent was obtained from the parents/guardian or patients following the relevant national regulations and institutional policies.

ORCID ID
C.C. Harish: 0000-0002-8780-8971
A.S. Shati: 0000-0003-0444-5595
S. Anandalakshmi: 0000-0001-5543-5130
A. Ahmad: 0000-0002-2634-5846
A.A. Ali: 0000-0001-9858-9789
R.M. Ravishankar: 0000-0003-4229-5778
K. Ashish: 0000-0001-7994-7130
A.A.M. Riyad: 0000-0002-6208-6714
A.H. Ahmad: 0000-0003938-5182
C. Kumarappan: 0000-0002-7981-4562.

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