Evaluation of frequency of autoimmune hepatitis autoantibodies in children with type 1 diabetes

Frecuencia de anticuerpos de hepatitis autoinmune en niños con diabetes tipo I

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ABSTRACT

Background: Diabetes mellitus type 1 (T1DM) is one of the childhood diseases with growing prevalence. Various accompanying autoimmune diseases were seen with type 1 diabetes. The most common autoimmune diseases with T1DM are autoimmune thyroiditis and celiac disease. In some reports, autoimmune hepatitis has been reported in association with DM-1. **Objectives:** The aim of this study was to evaluate autoimmune hepatitis autoantibodies in children with T1DM. **Materials and methods:** In this cross-sectional study, 202 children with T1DM were evaluated (47.5% were males and 52.5% were girls). Liver enzymes, autoimmune hepatitis related autoantibodies such as anti-nuclear antibodies (ANA), anti-smooth muscle (ASMA) and anti liver and kidney microsomal antibodies (LKM-1) were measured. Liver ultrasound was done for participants and biopsy of liver was taken for children with increased echogenicity of the liver, hepatomegaly or elevated liver enzymes. Results analyzed by statistical software spss-16, Descriptive statistics and chi-square test, paired T-TEST. Level of less than 5% was considered statistically significant. **Results:** In 6 patients ANA and in 4 patients (2%) ASMA was positive,1 patient was ASMA positive but ANA negative. None of the patients were Anti LKM-1 positive. 3 patients had positive ANA and ASMA, and increased liver echogenicity on ultrasound simultaneously. Histological evaluation was showed that 2 patients had findings in favor of autoimmune hepatitis. **Conclusion:** Auto antibodies were positive in 10 cases. ANA was positive in 6 (2.97%) of all cases. ASMA was positive in 4 (1.98%) cases. Increased echogenicity was found in 3 cases. Histological evaluation showed 2 patients had biopsy confirmed autoimmune hepatitis. AIH-2 was not seen among our cases.
Keywords: *Antibodies, antinuclear; Autoantibodies; Autoimmune diseases; Hepatitis (source: MeSH NLM).*

RESUMEN

Antecedentes: La diabetes mellitus tipo 1 (DM1) es una de las enfermedades infantiles con mayor prevalencia. Se observaron varias enfermedades autoinmunes acompañantes con diabetes tipo 1. Las enfermedades autoinmunes más comunes con DM1 son la tiroiditis autoinmune y la enfermedad celíaca. En algunos reportes, se ha encontrado hepatitis autoinmune en asociación con DM-1. **Objetivos:** El objetivo de este estudio fue evaluar los autoanticuerpos de hepatitis autoinmunes en niños con DM1. **Materiales y métodos:** En este estudio transversal, se evaluaron 202 niños con DM1 (47,5% eran hombres y 52,5% eran niñas). Se midieron las enzimas hepáticas, los autoanticuerpos autoinmunes relacionados con la hepatitis, como los anticuerpos antinucleares (ANA), el músculo liso (ASMA) y los anticuerpos microsomales hepáticos y renales (LKM-1). Se realizó una ecografía hepática para los participantes y se tomó una biopsia del hígado para niños con mayor ecogenicidad del hígado, hepatomegalia o enzimas hepáticas elevadas. Los resultados fueron analizados por el software estadístico spss-16 usando estadística descriptiva y prueba de chi-cuadrado, T-TEST pareado. Se consideró estadísticamente significativo un nivel menor del 5%. **Resultados:** En 6 pacientes con ANA y en 4 pacientes (2%) ASMA fue positiva, 1 paciente fue ASMA positiva pero ANA negativa. Ninguno de los pacientes fue anti LKM-1 positivo. 3 pacientes tuvieron ANA y ASMA positivas, y aumentaron la ecogenicidad hepática en la ecografía simultáneamente. La evaluación histológica mostró que 2 pacientes tenían hallazgos a favor de la hepatitis autoinmune. **Conclusión:** Los autoanticuerpos fueron positivos en 10 casos. ANA fue positivo en 6 (2,97%) de todos los casos. La ASMA fue positiva en 4 (1,98%) casos. Se encontró mayor ecogenicidad en 3 casos. La evaluación histológica mostró que 2 pacientes tenían biopsia confirmada de hepatitis autoinmune. AIH-2 no fue visto entre nuestros casos.
Palabras clave: *Anticuerpos antinucleares; Autoanticuerpos; Enfermedades autoinmunes; Hepatitis (fuente: DeCS BIREME).*

INTRODUCTION

Type 1 diabetes mellitus is one of the most common chronic diseases of among children. Despite the fact that almost 25 percent of cases are diagnosed in adulthood, this disease remains as the most common pediatric complication [1,2].

The incidence of type 1 diabetes in children varies based on the geographical location, age, gender, race

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and family history. The first peak of the disease happens during 4 to 6 years old and the second one appears during adolescence (10 to 14 years old) [3,4].

Both genetic and environmental factors are effective on the incidence of childhood diabetes. It has been reported that in children with family background of diabetes, the incidence of diabetes has increased significantly [5,6].

As mentioned before, diabetes type 1 appears alongside with a range of other diseases including autoimmune thyroiditis, celiac and Adison disease. In some cases, autoimmune hepatitis has been detected in association with diabetes type 1. The frequency of the antibodies associated with diabetes type 1 in autoimmune hepatitis has been reported as approximately 60% in Island cells antibody and 18.5% in insulin antibody [7,8].

Autoimmune hepatitis (AIH) is a progressive inflammatory liver disorder preferentially affecting females and characterized serologically by high amino-transferase levels, and presence of auto antibodies and histologically by interface hepatitis in the absence of a known etiology. Autoimmune hepatitis if left untreated may lead to liver cirrhosis and finally liver transplantation [9-11]. According to Verma *et al*. study, the worldwide prevalence of AIH was 2 to 17 per 100,000 children [12].

Auto immune hepatitis is divided into two types according to the auto antibody profile: patients with type I are positive for antinuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA), patients with type II are positive for anti-liver/kidney microsomal (LKM-1) and anti-LC-1 antibodies [7,13,14]. Diagnosis of autoimmune hepatitis was made using combination of laboratory and histological findings [15].

To determine the association between AIH and T1DM, Al-Husseini and colleagues conducted a study about autoimmune hepatitis related autoantibodies in Saudi Arabia in 2014. In their study, 106 patients were recruited for the study of their autoimmune hepatitis [8]. It was observed that 8 patients (7.5%) had a positive ANA test and one patient had positive anti LKM-1 test results. None of the patients in this study had organomegally, abnormal liver ultrasound or abnormal liver enzyme nor had biopsy. The researchers concluded that the presence of these antibodies in type 1 diabetes is rare and needs further related studies [8].

Autoimmune hepatitis still remains a major diagnostic and therapeutic challenge. Autoimmune hepatitis is particularly aggressive in children and progresses rapidly unless immunosuppressive treatment is started promptly. With early diagnosis and treatment, 80% of patients achieve remission and long-term survival [16,17]. There was limited published studies to evaluate association between T1DM and autoimmune hepatitis in children. The aim of this study was to evaluate autoimmune hepatitis and its related autoantibodies in children with T1DM.

MATERIALS AND METHODS

In this cross-sectional study, 202 children referred to, or those who were hospitalized in pediatric teaching hospital of the city Shiraz, in southwest of Iran, with diagnosis of type I diabetes were studied, in period of time from April 2014 to April 2015. Children, who had underlying disease affecting the liver activity, were excluded.

At First, a series of demographic information such as age, sex, duration of diabetes of those asked were recorded. Previous medical history, signs and symptoms associated with autoimmune diseases were recorded. Clinical examination was performed for evaluation of liver involvement.

Liver enzymes (AST: aspartate aminotransferase, and ALT: alanine transaminase), antibodies associated with autoimmune hepatitis, such as anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA) and antibodies against liver and kidney microsomal (LKM-1) patients were measured. A titer of anti-nuclear antibody $\geq$1/40 was considered positive and titer of $<$ 1/40 was considered negative. Anti-liver kidney microsomal antibody titer of $<$ 3 U/ml was considered negative, 3 - 5 U/ml borderlines, and $>$ 5 U/ml was considered positive. Tests in patients with positive results were repeated twice, and if the results were in the same meaningful laboratory range, were considered as positive. AIH-1 was considered if ANA or ASMA was positive [13,14,18,19]. AIH-2 was considered if Anti-LKM or Anti-LC was positive [13,14]. Liver ultrasound was done for participants. Patients with positive AIH–related auto-antibodies but normal ALT and AST and no abnormal finding on ultrasound were not candidates for liver biopsy but were followed up regularly in the clinic with repeated functional liver tests and autoantibody every 6 months. As these results were out of our study time-frame, further outcomes do not mention. Data were analyzed using SPSS software 16 (Chicago, IL, USA).

RESULTS

In this study, 202 children with diabetes were evaluated. People participating in the study included 96 patients (47.5%) male and 106 (52.5%) were female. The difference in sex was not significant in the present study ($p<0.05$). The mean age of the subjects was 10.38 $\pm$ 3.9 years old (ranged in age from 16 months to 22 years) were evaluated. The mean duration of disease

Table 1. Findings in patients with positive antibody.

patient	age	sex	AST	ALT	ANA	ASMA	Anti LKM	Physical exam	sonography	biopsy
1	5	F	51	29	pos	neg	neg	normal	normal	---
2	12	F	17	15	pos	neg	neg	normal	normal	---
3	4.5	M	30	18	neg	pos	neg	normal	normal	---
4	13	F	15	8	pos	neg	neg	normal	normal	---
5	6	M	178	143	pos	pos	neg	normal	Increase echogenicity	AIH
6	12	F	138	140	pos	pos	neg	normal	Increase echogenicity	AIH
7	15	F	26	27	pos	pos	neg	normal	Increase echogenicity	neg

T1DM: Type 1 diabetes; ANA: Anti-nuclear antibody; ASMA: Anti-Smooth muscle antibody; Anti-LKM: Anti-liver kidney microsomal antibody; F: Female, M: Male; AIH: Autoimmune hepatitis.

was 28.98 months. The average body mass index was 16.35 ± 3.2 kilogram per square meter.

Among 202 children, ANA in 6 (2.97%) patients was positive. Ds-DNA as a lupus index was investigated in these patients and this index in all of them was negative (Table 1).

ASMA was reported positive in 4 (1.98%) patients. None of the participants had a positive anti LKM-1. In one patient ASMA was positive but ANA was negative. The results of the study showed that 7 patients had positive antibodies for autoimmune hepatitis: 5 cases were females and 2 cases were males. This difference was statistically significant ($p<0.05$). The patients with positive autoantibodies (7 patients) did not have a significant statistical difference with respect to increase in age and duration of diabetes.

On the other hand, 5 patients who had the positive antibody had normal liver enzymes. The test results of the remained two patients who had elevated enzymes of the liver biopsy also supported autoimmune hepatitis.

Three patients had positive ANA and ASMA simultaneously. All of these three patients had an increased liver echogenicity by ultrasound study. Liver biopsy was performed for these three patients. Autoimmune hepatitis were reported in histological examination of two samples. The rest of the participants (4 patients) who had positive antibody results, due to their normal ultrasound and clinical examination and the absence of increased liver enzymes, did not need liver biopsy and liver biopsy were not performed. Children with confirmed autoimmune hepatitis underwent treatment.

Ethical approval: This study was approved by ethical committee of the university.

Informed consent: Informed consent was obtained from all individual participants included in the study in Persian language. And no identifying information about participants is available in the article.

Limitation: Single center study.

DISCUSSION

Diabetes is a common childhood disease. Due to its relation with autoimmune factors, the disease association with a number of autoimmune diseases has been investigated and reported. Diabetes type 1 is associated significantly with autoimmune diseases. The most common of these diseases are thyroiditis (30%), celiac (4-9%) and Addison disease (0.5%) [8].

Autoimmune liver disease that is often associated with diabetes is autoimmune hepatitis type II. The prevalence of auto-antibodies which related to diabetes mellitus in autoimmune hepatitis was reported 60.7% for island cells antibodies and 18.5 for insulin antibodies respectively [8].

Allen *et al*. had screened adults with T1DM for presence of organ specific autoantibodies; 2 out of 261 (0.8%) were positive for SMA and none was positive for LKM-1 antibody [20]. In the current study, LKM-1 was not positive among all cases.

In a controlled study in adults, Heras *et al*. found that 19 of 70 patients (27%) with T1DM had positive ANA, as compared to 4 of 28 patients with type 2 diabetes (14%) and 4 of 20 healthy controls (20%) [21]. The findings of the present study show that 6 (2.97%) patients among 202 participants had positive tests for ANA, that three had ASMA at the same time. One person alone had a positive ASMA. In the study by Al-Hussaini *et al*. 8 out of 106 participated patients with T1DM were ANA positive, one anti-LKM 1 positive, and none of them were ASMA positive and none of their patients had elevated liver enzymes [8]. And they could not conclude the clinical significance of these autoantibodies in children with T1DM [8]. ANA seropositivity was lower in our study compared to Al-Hussaini *et al*. study [8]. Heras *et al*. study was conducted in adult cases [21].

In the study by de Sousa *et al* on the national registry, prevalence of AIH in patients with T1DM was 44.8 per 100,000 cases [22]. It seems rate of ANA seropositivity was

Table 2. Summary of Seropositivity of Autoimmune Markers in patients with T1DM reported by published studies.

Study	Sample size (T1DM cases)	Seropositivity of autoimmune marker
Current study (children)	202	LKM=None ANA=2.97% SMA=1.98%
Al-Hussaini *et al.* [8] (children)	106	LKM=1% ANA=7.5% SMA=Neg
Allen *et al.* [20] (adult cases)	327	LKM=None SMA=0.8%
Heras *et al.* [21] (adult cases)	70	ANA=27% SMA=1.42%

higher in T1DM adult cases compared to children cases (Table 2). The summary of studies was shown in Table 2.

In our study it was observed that autoantibodies are more in females than in males that these findings were consistent with other studies in this field [8]. As mentioned in the previous published articles, AIH more commonly affects female more than male [23].

It is possible that autoimmune dysfunction in patients with T1DM could have contributed to ANA reactivity, as systemic non-liver autoimmune diseases, like systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome or other connective tissue diseases, are a well-recognized causes of ANA positivity [16]. However, it seems unlikely that any of the ANA-positive patients in this study have sub-clinical systemic lupus erythematosus as all were anti-double stranded DNA negative.

Although In this study, the association of autoimmune hepatitis with type 1 diabetes has been slightly detected, it could be considered of great importance due to the high significance of this association. It should be noticed that the 2 patients with histologically proven AIH in our study were both ANA and ASMA positive with elevated liver enzymes.

In conclusion, the frequency of AIH in T1DM children was very low. It seems routine testing for autoimmune hepatitis among children with T1DM is not appropriate except. Also, future studies should consider testing for complete panel of AIH-related autoantibodies such as anti-soluble liver antigen and anti-liver cytosol type 1 [17].

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Conflict of Interest: There was no conflict of interest upon this study.

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