

## A Comparative Study on Enteroviruses in Blood and Stool of Newly Diagnosed Children with Type 1 Diabetes Mellitus and Healthy Children

\* Sepideh Bagheri <sup>1</sup>, Nosrat Ghaemi <sup>1</sup>, Samaneh Noroozi Asl <sup>1</sup>, Mohammad Hassan Aelami <sup>1</sup>, Saeed Amel Jamehdar <sup>2</sup>, Mojtaba Lotfi <sup>1</sup>, Samira Asli <sup>2</sup>, Reyhaneh Elmi Sangdivar <sup>3</sup>

<sup>1</sup> Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>2</sup> Department of Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>3</sup> Pediatric Endocrinology Ward, Akbar Children's Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

### Abstract

**Background:** Type 1 diabetes mellitus results from autoimmune destruction of the insulin-producing beta cells in the islets of Langerhans in pancreas. It is one of the most common chronic diseases in childhood. This process occurs in genetically susceptible subjects and is probably triggered by one or more environmental agents. Viral agents especially enteroviruses are under special focus as the environmental triggering factors and many studies all around the world have evaluated their role in the pathogenesis of type 1 diabetes. As the prevalence of viral infections varies in different geographical regions, this study was planned to evaluate the frequency of enterovirus infection in the population of diabetic children in North East of Iran.

**Methods:** 35 children between 1 to 18 years who were newly diagnosed with type 1 diabetes were investigated for enterovirus RNA using RT-PCR in their serum and stool, and 31 nondiabetic children who were also negative for other autoimmune disorders were also evaluated.

**Results:** Enterovirus RNA RT-PCR was negative in serum of all case and control samples. Stool samples were also negative in the case group and only one stool sample was positive in the control group. The patient was totally asymptomatic and had received polio vaccine 2 weeks before.

**Conclusion:** Results of this study show that presence of enterovirus RNA at the time of the clinical presentation of diabetes is not common in our population of diabetic patients and more extensive studies are needed to establish a relationship.

**Key Words:** Children, Enterovirus, RT-PCR, Type 1 diabetes.

\* Please cite this article as: Bagheri S, Ghaemi N, Noroozi Asl S, Aelami MH, Amel Jamehdar S, Lotfi M, Asli S, Elmi Sangdivar R. A Comparative Study on Enteroviruses in Blood and Stool of Newly Diagnosed Children with Type 1 Diabetes Mellitus and Healthy Children. Int J Pediatr 2023; 11 (08):18157-18162. DOI: **10.22038/ijp.2023.73844.5329**

### \*Corresponding Author:

Sepideh Bagheri, Department of pediatrics, Faculty of medicine, Mashhad University of medical Sciences, Mashhad, Iran. Email: bagheris@mums.ac.ir

Received date: jul.19,2023; Accepted date: Aug.18,2023

## 1- INTRODUCTION

Type 1 diabetes mellitus is one of the most common chronic diseases of childhood (1). It is characterized by the autoimmune destruction of pancreatic beta cells. It occurs in genetically susceptible individuals and is most probably triggered by environmental factors like nutrition and infections (2).

Epidemiological studies have shown some seasonal and geographical variations in the incidence of type 1 diabetes and this has led to the hypothesis that viral infections may play a role in the pathogenesis of type 1 diabetes (3).

Enteroviruses are largely accused for their role in triggering autoimmunity and beta cell destruction and have been extensively studied worldwide (4, 5). Among enteroviruses, Coxsackie Virus B (CVB) is mostly linked to the development of type 1 diabetes (6, 7). Most CVB infections are asymptomatic or have few symptoms but they may cause meningitis, myocarditis, encephalitis, hepatitis and pancreatitis as well (8).

The exact mechanism by which enteroviruses induce autoimmunity against beta cells of pancreas are not yet fully understood. Detection of enterovirus RNA in the serum and in pancreatic beta cells of patients with diabetes type 1 and also positive anti-CVB antibodies in sera of these patients have supported the hypothesis of persistent enterovirus infection as a possible mechanism for the progressive destruction of pancreatic beta cells (9, 10).

In some previous studies, enterovirus RNA has been isolated from the peripheral blood of patients at the onset of type 1 diabetes (11-13).

Because viral infections have geographical variation and their incidence varies among different countries, this study was performed to evaluate the presence of

enterovirus RNA using real-time PCR (RT-PCR) in serum and stool of newly diagnosed children with type 1 diabetes in comparison to those of their health counterparts in north east of Iran.

## 2- MATERIALS AND METHODS

This was a cross sectional case control study. 35 children between 1 to 18 years who were newly diagnosed with type 1 diabetes, according to the criteria specified by the International Society for Pediatric and Adolescent Diabetes (ISPAD), and had at least two positive auto antibodies of type 1 diabetes were investigated for the presence of enterovirus RNA using RT-PCR in their serum and stool and 31 nondiabetic children who were also negative for other autoimmune disorders were evaluated as control group. The study was performed at the pediatric endocrinology ward of Akbar children's hospital in Mashhad, North east of Iran. This hospital is affiliated with Mashhad University of Medical sciences and is a referral center for pediatric endocrinology in north east of Iran.

A checklist was used for gathering demographic and anthropometric data. 2 milliliters of citrated blood and a stool sample were collected for each patient in the case and control groups. RNA was extracted from 140  $\mu$ l of the serum or fecal suspension using AmpliSens® Human Enterovirus-FRTPCR H-2773-1-CE kits.

### 2-1. Data Analysis

All data were analyzed using the SPSS 22 statistical package. Data were presented using descriptive statistics including means, standard deviation and proportions. Student's T-test was implemented for quantitative data and chi-square test for qualitative data. Kolmogorov-Smirnov test was used to test the normality of the independent variables. Level of significance in terms of P-value was considered 0.05, for all parameters.

### 3- RESULTS

The descriptive findings revealed that 51.4% of cases in the diabetic group were boys and 48.6% were female. This was 58.1% and 41.9% in the control group and no significant difference was observed between the two groups regarding their sex ( $p$  value=0.58). Mean age of patients was  $7.38 \pm 2.95$  years in the case group and  $6.71 \pm 3.88$  years in the control group. The two groups did not differ significantly regarding their age ( $p = 0.98$ ). 85.7% of diabetic patients were living in cities and 14.3% in rural areas. This was 90.3% and 9.7% in the control group, respectively ( $P=0.56$ ).

Plasma enterovirus RT-PCR was negative in all patients of both case and control groups. Stool specimens were also all negative in diabetic group and only one stool specimen was positive for enterovirus in the control group. She was an 18 month old asymptomatic child who had received oral polio vaccine 2 weeks before.

### 4- DISCUSSION

In the present study 35 newly diagnosed children with type 1 diabetes and 31 healthy children were evaluated for the presence of enterovirus RNA in their plasma and stool using RT-PCR. Enterovirus RNA RT-PCR was negative in the serum of all case and control samples. Stool samples were also negative in the case group and only one stool sample was positive in the control group. The patient was totally asymptomatic and had received polio vaccine 2 weeks before.

Studies from several countries have observed anti-enterovirus antibodies and/or enterovirus RNA more prevalently in the blood of newly diagnosed type 1 diabetic patients than in healthy controls (14-17).

In a study by Sarmiento et al. in Cuba where the incidence of type 1 diabetes is

low but the circulation of enteroviruses are high, it was found that enterovirus infections are associated with T1DM onset and  $\beta$ -cell autoimmunity (18). Their study showed a significantly higher frequency of enterovirus RNA in serum from T1DM children at the onset of disease, as well as in their ICA positive first-degree relatives compared to healthy control subjects and the patients' ICA negative first-degree relatives.

Considering the fact that our study was coincident with the pandemic of Covid 19 and this was the time that schools and daycares were locked down and children were taught to consider self-hygiene strategies like using hand rub and face masks, the incidence of other viral infections declined significantly and enterovirus infection transmission should have decreased too. But if we accept that they play a role in the pathogenesis of type 1 diabetes, we should expect that the incidence of type 1 diabetes declines among children; but such an observation is not documented and there are reports from some parts of the world, that the incidence of type 1 diabetes among children has increased during the Covid 19 pandemic (19-21). This finding shows that other factors may have played a role in the pathogenesis of type 1 diabetes in these children; and one possible hypothesis is the role of covid19 virus as a triggering factor for autoimmune destruction of pancreatic beta cells (22-24). Another possibility for not detecting any enterovirus RNA in our population of newly diagnosed pediatrics with type 1 diabetes might be the fact that because of the fear of covid 19 infection, the patients sought medical attention very late and mean Hemoglobin A1c in our study sample was 10.9% which shows that they had a long duration of hyperglycemia before diagnosis.

In a study by Alidjinou et al., (25) the researchers detected enterovirus RNA in

only one sample out of 13 using RT-PCR. But when they used integrated cell culture, more positive results were yielded. This shows that using other techniques besides RT-PCR may result in more positive samples.

In a meta-analysis about the association between enterovirus infection and diabetes type 1, 38 studies including 5921 patients (2841 type 1 diabetes cases and 3080 controls) were evaluated. Their findings suggested that enterovirus infection is associated with T1DM (26). In this study no association was found among stool samples. They concluded that this is due to the chronicity of the infection and shows that the infection is not acute. In our study, no positive stool samples were obtained.

#### **4-1. Limitations of the Study**

The results of this study are limited by its rather small study population. Multicenter studies with larger study populations are, then, recommended. Applying other diagnostic tests, like integrated cell culture, may also yield more positive results; and should be considered in future studies in the field.

#### **5- CONCLUSION**

We could not support the association between enterovirus infection and type 1 diabetes in our population of diabetic children at the onset of the disease. Multi center studies with a larger population are needed in our region in order to rule out this association. Use of supportive laboratory tests like integrated cell culture or checking enterovirus antibodies in serum of diabetic children can also help.

#### **6- ETHICAL CONSIDERATIONS**

This study was approved by the medical ethics committee of Mashhad University of Medical Sciences (MUMS) prior to performance and all patients or their guardians signed informed consent forms.

#### **7- CONFLICT OF INTEREST**

None

#### **8- ACKNOWLEDGMENTS**

This article was based on the thesis of Dr Sepideh Bagheri with the thesis number T-5962. We kindly appreciate the assistance of the clinical research Development Unit of Akbar Hospital in performing this research. We thank our patients and their families for their cooperation.

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