

# Type 1 diabetes mellitus and enterovirus linkage: search for associated etiopathology

Idris Abdullahi Nasir<sup>a,b</sup>, Anthony U. Emeribe<sup>e</sup>, Halima A. Shuwa<sup>f</sup>,  
Maryam M. Zakari<sup>c</sup>, Nkechi Onukegbe Peters<sup>d</sup>

<sup>a</sup>Department of Medical Microbiology and Parasitology, University of Ilorin, Ilorin,  
<sup>b</sup>Department of Medical Laboratory Services,  
<sup>c</sup>Department of Obstetric and Gynaecology, University of Abuja Teaching Hospital, Gwagwalada, <sup>d</sup>Department of Strategic Information and Research, Institute of Human Virology, Abuja, <sup>e</sup>Department of Medical Laboratory Science, University of Calabar, Calabar, <sup>f</sup>Department of Immunology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria

Correspondence to Idris Abdullahi Nasir, MSc, Department of Medical Laboratory Services, University of Abuja Teaching Hospital, PMB 228 Gwagwalada, Abuja, Nigeria;  
Tel: +234 802 285 2352;  
e-mail: eedris888@yahoo.com

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Type 1 diabetes (T1D) is believed to have complex interplay between several enteroviruses (EVs) and host immune system disturbance induced or accelerated by viral pathogenesis. In the past two decades, there has been global upsurge in the incidence of childhood T1D, especially in those less than 5 years. Because of the ubiquity and persistence of EVs in human bowel and their tropism to pancreatic cells, they tend to express certain viral proteins that have propensity for genetic manipulation and activation of autoimmunity that could be potentially linked to T1D. In view of these, we present this review of existing literature in order to analyze the epidemiology and possible association between EV infections, host immune dysfunction, and development of autoimmunity or T1D with the view to encourage the investigation of EV infections and associated virus-induced islet cells autoimmunity and immunopathy in genetically predisposed children.

## Keywords:

diabetes, enterovirus infection, immunopathology

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## Introduction

Type 1 diabetes (T1D) is one of the most common chronic diseases in developed countries and represents about 10% of all cases of diabetes mellitus. T1D is the result of a selective autoimmune destruction of pancreatic islet  $\beta$  cell of Langerhans, occurring in genetically predisposed individuals, possibly triggered or accelerated by environmental agents [1]. In addition, innate and adaptive immune responses are involved in islet inflammation in T1D. In the past three decades, several studies have searched for environmental triggers, but it has remained difficult to define the key players [2]. In addition to viral infections, hygienic and nutritional condition, as well as sunlight exposure and geographical locations, have all been investigated but often with inconclusive results [3]. In 2011, Kingdom of Saudi Arabia had the highest cases of T1D in 10 000 children between 1 and 14 years; similar figures were recorded in Northern American and Pacific regions. However, there is paucity of data in regard to T1D in Sub-Saharan Africa children (Fig. 1) [4].

Human enteroviruses (EVs) are small nonenveloped RNA viruses that belong to the Picornaviridae family; the genus *Enterovirus* currently encompasses seven species involved in human diseases (human EV A–D

and human rhinovirus A–C). EVs include many major human pathogens such as poliovirus, rhinovirus, EV 71, coxsackievirus, and echovirus [5]. Most EVs are stable in media with pH from 3 to 9. They can be resistant to chemotherapy, to antibiotics, to 70% alcohol, and to some detergents. They are, however, thermolabile and are destroyed if exposed to temperatures above 50°C [6].

## Epidemiology of enteroviral infections in type 1 diabetes patients

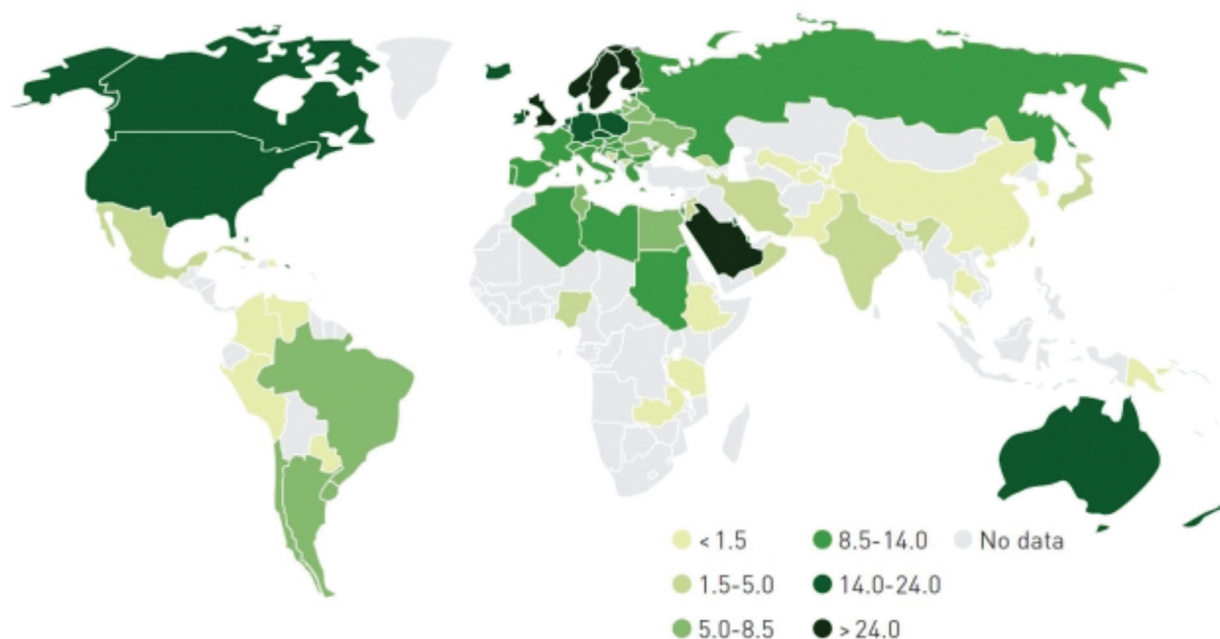
The association between EV infections and T1D has gained wide interest from several epidemiological studies. Although genetic predisposition to T1D has been confirmed, the environmental triggers of the disease have not been proven. Several studies point to the involvement of EVs such as coxsackievirus A, coxsackievirus B, and echovirus to T1D [7].

In 1969, Gamble *et al.* [8] were the first to provide evidence of possible linkage between EV infection and T1D. Reports of the involvement of coxsackievirus B infections in the pathogenesis of T1D were later made

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Figure 1

Map 2.5. New cases of type 1 diabetes (0-14 years per 100,000 children per year), 2011



Global incidence of type 1 diabetes [4].

available [9], yet several other EV species may also have links with T1D.

Cases of new-onset T1D occur in seasonal patterns [10], sometimes in clusters outbreaks, often peaking 1–2 months after high EV infection activity [11]. An intrafamilial spread of EV infections showed that 20% of siblings of diabetic probands acquired T1D with a latency of 3–25 months [12]. Evidence for this association is supported by detection of EVs in the blood, pancreas, and gut mucosa of patients with T1D [13–15]. Histopathological findings indicated that expression of enteroviral capsid protein VP1 in islet cells of patients with T1D is related to induction of protein kinase R and downregulation of MCL-1 [14]. A recent cohort meta-analysis of molecular studies confirms the association between human EV infection and T1D [7].

#### Mode of transmission of enteroviruses

EVs can be found in an infected person's stool, nose, eyes, and oral secretions (such as sputum and saliva), or blister fluid [16]. People get exposed to EVs by having close contact, such as shaking hands with an infected person, touching surfaces or objects that have the virus on them, then touching your eyes, nose, or mouth before washing your hands, changing diapers of an infected person, then touching your eyes, nose, or

mouth before washing your hands, or drinking water that has EVs [16].

Pregnant women infected with EVs before delivery can pass the virus to their fetuses. In addition, mothers who are breastfeeding should talk to their physicians if they are sick or think they might have been infected [16]. EVs can be shed (passed from a person's body into the environment) in feces for several weeks or longer after being infected. The virus can be shed from the respiratory tract for less than or equal to 3 weeks. Importantly, infected people can shed EVs even if they do not have symptoms [16]. Consequently, the information regarding the detection of EVs in water, such as epidemiological data related to waterborne diseases, is crucial to modern public health systems [17].

#### Association of enteroviruses and type 1 diabetes mellitus

EVs have traditionally been implicated in the etiopathogenesis of T1D with no definitive proof so far. However, EVs are one of the main candidates because traces of viral infections have been found more frequently in patients with T1D than in individuals without diabetes [7]. This does not directly prove that EVs cause T1D; they could also be mere secondary players in a complex immune milieu that ends with the destruction of pancreatic  $\beta$  cell of Langerhans. EVs

have tropism to pancreatic islets and can cause  $\beta$ -cell damage in experimental models. In addition, viral persistence has been suspected to be an important pathogenetic factor in T1D [2].

As the understanding of the pathogenesis of T1D requires focusing on the pancreas and pancreatic tropism of EVs, the concept of potential viral presence needs to be defined. In support to this concept, the demise of  $\beta$  cell of Langerhans, elevated levels of MHC-I have been found in donors with T1D, consistent with a possible virus-induced interferon mediated signal facilitating antigen presentation to infiltrating cells [18]. Some studies have found a stronger interferon signature in individuals at high risk of T1D [19]. Upregulation of MHC-I could then explain ensuing events, such as the infiltration of autoreactive CD8 T lymphocytes (that are also increased in blood from patients with T1D) and the selective loss of pancreatic  $\beta$  cell of Langerhans [20].

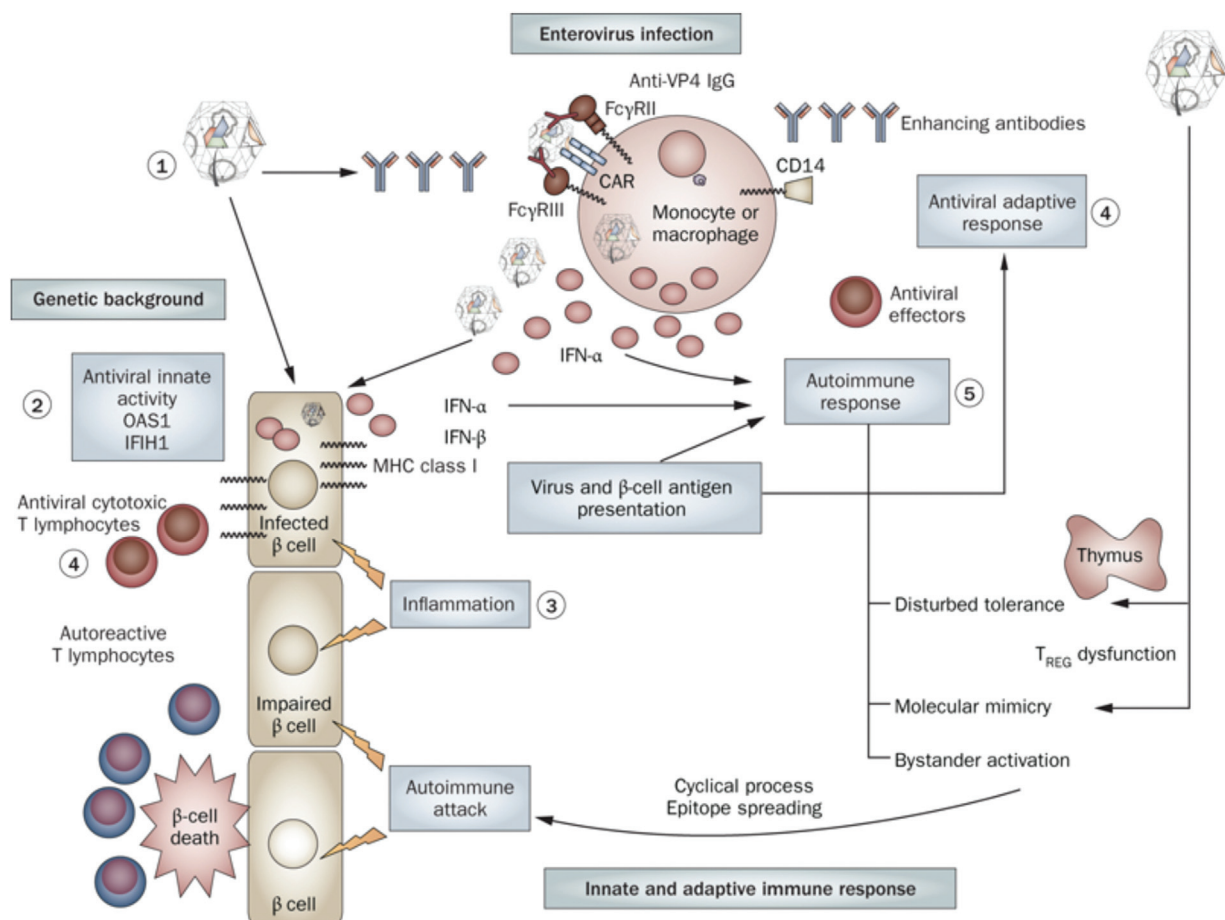
In humans, the evidence of enteroviral infection within pancreatic cells at the onset or during the progression of T1D has been difficult to obtain as this requires a

biopsy that is invasive and often risky. Therefore, most of the data available come from necropsies [14]. Pancreatic islets and especially  $\beta$  cells, but not exocrine cells, were found to be susceptible to enteroviral infection [21,22] (Fig. 2).

Interestingly, the specific receptor of coxsackieviruses, the CAR molecule, is expressed in the pancreas mainly by  $\beta$  cells [24]. Coxsackie virus B (CVB) can effectively replicate in pancreatic cells and cause massive cell lysis [25]. Things are different in CVB-associated autoimmune T1D, as a clinical disease occurs often many years after the appearance of islet specific autoantibodies, which have been reported to be as result of enteroviral infection [25].

As the selective destruction of  $\beta$  cells in T1D patients is an autoimmune process, the main hypothesis addressing the relationship between CVB persistence and T1D is that noncytopathic CVB infection triggers autoimmunity against  $\beta$  cells through activation of inflammation. Further pathological studies on pancreases from dead T1D patients revealed a quasi-absence of  $\beta$  cells and the presence of an inflammatory

Figure 2



Mechanism of enterovirus pancreatic cell destruction and associated immunopathology [23].

cell infiltrate (insulinitis) composed mainly of CD8 cytotoxic T cells and to a less extent CD4 T cells and macrophages, and sometimes NK cells were reported [22,26].

Other non-EVs that can promote autoimmunity and diabetes in rats or mice have been studied. For instance, rotavirus has been shown to accelerate diabetes in the nonobese diabetic rats in an age-dependent manner [27]; the presence of endogenous retrovirus has been reported to correlate with diabetes susceptibility and progression [28]; Mengovirus causes diabetes in addition to encephalitis, which led to islet necrosis [29]; and Ljungan viral particles detected in wild-trapped bank voles and biobreeding rats were reported to induce autoimmunity [30].

Cross-reactivity is a common serological feature of certain human viral infections such as Epstein-Barr virus, hepatitis C virus, hepatitis A virus, and HIV, and persisting viral infection has long been shown to lead to polyclonal B-cell activation [31], which would be the crucial prerequisite for autoimmunity especially in T1D comorbid patients [32].

#### **Pathogenesis of enterovirus-induced pancreatic B-cell destruction**

The human gut has crucial roles in the development of most immune-mediated disorders. Besides absorbing nutrients, intestinal epithelial cells also contain Toll-like receptors and other signaling mechanisms for the transduction of inflammatory signals from the intestinal lumen [13]. Many studies suggest that the gastrointestinal tract (GIT) has an important role in the pathogenesis of T1D [13]. Intestinal microfloras, permeability of mucosal barrier, and immunity have all been linked to T1D [33]. Environmental factors can modify the normal gut microflora or they can change the gut cytokine milieu, including gastrointestinal tract infections [34].

The mechanisms by which EVs may contribute or cause destruction of  $\beta$  cells are not fully understood [23]. Animal models on virus-induced diabetes suggest that viruses can infect and destroy  $\beta$  cells directly or induce an immune-mediated process leading to  $\beta$ -cell damage [35]. Direct viral effects could be mediated by induction of apoptosis or necrosis in infected cells, as well as impaired function of  $\beta$  cells through shut-off of cell functions.

One possibility is that EV infection leads to an autoimmune process and destruction of  $\beta$  cells by so-called bystander activation mechanism [36].

This theory implies that local virus infection in the pancreatic islets maintains inflammation and creates proinflammatory milieu leading to activation of antigen-presenting cells and autoreactive lymphocytes [37]. Another mechanism by which EVs could cause  $\beta$  cell destruction is molecular mimicry, when autoimmune attack results from immunological cross-reactivity induced by similar structures in the virus and  $\beta$ -cell autoantigens [38].

Studies with isolated human pancreatic islets cells of Langerhans have demonstrated that the effect of virus replication in the islet cells is serotype and strain-dependent, as some of the viruses cause severe morphological changes and cell death, as well as functional impairment, whereas some serotypes and isolates are less destructive [39]. These islet cell experiments have demonstrated that most of the studied EVs infect predominantly  $\beta$  cells, although some EVs seem to infect both  $\beta$  and  $\alpha$  cells [40].

The genetic background of the host influences the development of  $\beta$  cell damage and diabetes in response to a viral infection [41]. Several genes that are associated with T1D are known to have a role in antiviral immune responses. The Human Leukocyte Antigen genes that modulate the risk of T1D are associated with immune response against certain viruses including EVs [42] and can also modulate the course of virus infections [43].

Genetic sequence homology with an identical PEVKEK motif exists between coxsackie B4 and the islet antigen GAD65; this suggests the possibility of molecular mimicry [44]. However, findings of immune cross-reactivity are conflicting. Further suggestive evidence of acute versus persistent EV infection comes from cytokine studies in the newly diagnosed T1D patients. The most potent antiviral cytokines are interferon- $\alpha$  and interferon- $\beta$  [44]. These form part of the early innate response to viral infection, and high levels therefore suggest recent infection [44]. Autopsy reports have shown that interferon- $\alpha$  was expressed on the surface of  $\beta$  cells, but not other islet endocrine cells, in patients who died soon after diagnosis of diabetes [45]. Raised circulating levels of interferon- $\alpha$  have also been reported in the serum of newly diagnosed patients, but might simply reflect the proinflammatory milieu that exists at that stage of T1D [44,45].

#### **Prevention and treatment of enteroviruses infections**

Most people infected with EVs do not have clinical symptoms, but can still spread the virus to other people.



This makes it difficult to prevent them from spreading [46]. However, the best way to protect people from EV infections is to wash your hands often with water and soap, especially after using the toilet and changing diapers, and to avoid close contact, such as touching and shaking hands, with people who are sick. In addition, cleaning and disinfecting of frequently touched surfaces is very important in minimizing risk of contracting EVs from inanimate objects [46].

Even though there is no specific treatment for EV infection, as with other viral pathogens, there are several steps in the replication cycle of the EVs that are potential targets in antiviral therapy. Cell susceptibility, viral attachment, viral uncoating, viral RNA replication, and viral protein synthesis have all been studied as targets of antipicornaviral compounds [47].

As the primary mechanism of EV clearance is by humoral immunity, patients who lack antibody because of congenital or acquired immunodeficiencies are uniquely susceptible to infections with the EVs [48]. Similarly, normal neonates are at a high risk for severe EV disease because of a relative deficiency of EV antibodies [49]. Immune serum globulin has been used prophylactically and therapeutically against the EVs in two clinical settings: the neonate and the immunocompromised host [47,50]. Neonates may develop an overwhelming sepsis syndrome from transplacental/peripartum acquisition of EV infection. The high mortality rate of this disease, coupled with the known association of severe EVs associated diseases such as autoimmunity, has prompted numerous investigators to administer antibody preparations to neonates with EV sepsis [47,50].

There is currently no licensed vaccine to protect people from EV infection. However, there are vaccine candidates for EV-A71 in clinical trials. The inactivated EV-A71 vaccine is considered the safest viral vaccine [50]. Up to date, five organizations have completed preclinical studies to develop an inactivated vaccine that is at different phases of clinical trials [50]. Three of the companies are from China, whereas the other two are from Taiwan and Singapore, respectively. They have all completed Phase III Clinical Trials in 2014 for an inactivated EV-A71 vaccine against the subgenotype C4, as it was the main subgenotype responsible for outbreaks in China [50]. The three companies conducted randomized, double-blind, placebo-controlled, multicenter trials involving over 30 000 healthy children. Each candidate received two intramuscular doses of vaccine or placebo within a span of 28 days apart [51].

Sinovac reported that their inactivated vaccine efficacy was 94.8% and anti-EV-A71 immune response elicited by the two dose vaccines were found in 98.8% of participants [52]. In addition, the anti-EV-A71 neutralizing titer of 1 : 16 associated with protection against EV-A71 was expected to last for at least 1 year. However, the neutralizing antibody titer was found to decline by 50% after 6 months [52].

#### **Diagnosis of enteroviruses in diabetic patients**

Specific diagnosis of EV infections demands detection in samples collected from the patient, as clinical signs are not sufficiently specific because of the diversity of host responses and the large number of different EV serotypes. Diagnosis involves both clinical and epidemiological features [53]. The main methods used to detect EVs include viral culture, serology immunofluorescence, and nucleic acid amplification tests. Using two techniques in combination might be a good option to identify EVs and to associate it with the development of T1D, particularly in children [54]. In addition to epidemiological studies, an association between EVs and T1D has been evaluated by searching for EVs in the pancreatic tissue of T1D patients. Obviously, in case of viral persistence, the virus should be detectable for longer periods of time and at different stages of the diabetic process assuming that enough virus proteins and RNA are produced [15]. This means that the possible absence of the virus in T1D patients would not necessarily exclude the role of EV infections, which could have occurred earlier and started the  $\beta$ -cell damaging process but the virus had already disappeared by the time of clinical diagnosis of T1D [15].

Serological assays using EV-specific immunoglobulin M and immunoglobulin G have limited application in diagnosis of EV infection. This is because EV serology has poor technical indices of antibody detection and possibility of cross-reaction with similar viruses such as adenoviruses and reovirus [15].

The commonly used antibody-based test in EVs detection is immunohistochemistry (IHC). Recently, the possible cross-reactivity of antibodies used in IHC with pancreatic islet cell proteins has been studied extensively [21]. Altogether, these antibodies seem to react with several EV types. In addition, it may also recognize some host proteins if used in nonoptimal conditions, and therefore the staining conditions should be optimized for different tissues and the presence of the virus should be confirmed by other methods [15]. With the advent of molecular techniques, identification has become easier, which has helped both patient diagnosis and epidemiological studies.

## Conclusion

T1D is a public health issue that has affected population of all races with high healthcare-associated expenses on attempting to maintain patients' quality of life. In many countries, T1D has become a crucial noncommunicable disease that has been linked to several EV infections. PCR studies of viremia before or around the time of T1D diagnosis is quite significant, but is difficult to interpret. However, cohort prospective studies from birth using improved investigation technologies will eventually solve some of these issues. In view of these, there is a need to open new opportunities for the investigation of EV infections and associated virus-induced islet autoimmunity and immunopathy in young genetically predisposed children.

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## Conflicts of interest

There are no conflicts of interest.

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