

Case Report

Virological Investigation of Chronic Diarrhea in Patients with Primary Immunodeficiency

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Abstract

Primary Immuno Deficiencies (PID) are rare disorders accompanying with immune dysregulation and different types of infections. Chronic diarrhea is a common complication that occur in 20% of these patients. As causes of diarrhea are diverse and include various microbial agents; thus, the aim of this study was to investigate the virological status of chronic diarrhea in patients with PID referred to the Allergy and Clinical Immunology Department of Rasoul Akram hospital by PCR, endoscopy, and colonoscopy. We evaluated 54 patients with PID and these results were obtained: no virus was found in 42 (77%) patients and 12 patients (23%) had the viruses as the following: Norovirus was the most common virus detected in 4 patients (7.4%) and there was a patient with concomitant Norovirus and Cytomegalovirus. Colonoscopy and endoscopy were performed for the patients who were positive for Cytomegalovirus and this virus was associated with Norovirus and Enterovirus in other 2 patients. The results of colonoscopy showed that Cytomegalovirus was present in 3 patients (5.6%). In conclusion, we found a number of viruses causing chronic diarrhea in PID patients; therefore, appropriate treatment is applicable to reduce the long-term complications of these patients.

Keywords: Primary immunodeficiency; Diarrhea; Norovirus; Cytomegalovirus; IgG

Introduction

Primary immunodeficiencies (PID) are rarely found disorders related to immune system and the affected patients are predisposed to an elevated rate of severe infections, immune dysregulation accompanying with autoimmune disease, aberrant inflammatory responses, and malignancies [1]. The exact prevalence of PIDs in the world's population is uncertain; however, is estimated to affect 1 in every 2000 births. More than 200 genetic disorders affecting the immune system have been identified to date [2]. Most studies reported a significant reduction in infection occurrence in association with applying high doses of intravenous immunoglobulin (iv) therapy [3, 4]. A large population of patients with recurrent infections is undiagnosed or underdiagnosed and the extensive record of these patients has led to the recognition of more patients. According to the Jeffrey Modell Foundation, 60360 patients were registered by 2015 [5]. Diarrhea occurs in 20% of patients with PID. In fact, diarrhea is called to stool excretion more than 3 to 4 times a day and chronic diarrhea is referred to as diarrhea persistence for longer than 4 weeks [6-8]. Common Variable Immunodeficiency (CVID) is the most frequent symptomatic PID. CVID is characterized by low levels of IgG and IgA and/or IgM, normal or decreased B cell numbers, and impaired antibody response leading to recurrent infections noted mostly in the GI tract. Studies showed that the prevalence of GI complaints among patients with CVID is high [9] and between

7% and 23% of the CVID patients suffer from intractable diarrhea [10, 11]. The gastrointestinal tract (GI) is one of the largest immune organs in the body that directly and indirectly interacts with immune system [12]. Unlike other organs, it exists in a state of physiologic inflammation because of constant exposure to ingested foreign antigens. Therefore, it is not surprising that defects in the immune system may encounter GI to multitude microbial inflammations [13]. The hallmarks of PID include recurrent or persistent infections, infections due to microorganisms that rarely cause detectable disease in immunocompetent people. Recent studies reported that PID patients are susceptible a range of infections with one or several viruses, including EBV, Rotavirus, Picornavirus, Parvovirus, Papillomavirus, Echovirus / Enterovirus, and Herpes simplex virus [14, 15]. Although the frequency of PID patients is growing, its associated ambiguous aspects remain to be surveyed in these patients, especially virological compositions. Therefore, the aim of this study was to investigate of the virological status of chronic diarrhea in PID patients.

Methods

Patient selection

This study was the cross-sectional type. All the patients with PID who referred to the Allergy and Clinical Immunology clinic of Rasoul Akram hospital were evaluated from 2015 to

2016. At first, 76 patients with PID that were diagnosed based on the criteria of the PAGID (Pan American group of immune deficiency) and ESID (European society of immune deficiency) systems entered in the study. Then, patient information was recorded and finally, 54 patients with PID entered the study that of these, 26 women and 28 men with at least and most age of 4 months and 55 years were included. Patients were divided into three groups without diarrhea, chronic diarrhea (for more than 4 weeks) before and after the diagnosis of PID. The stool samples were taken from all patients and were kept at -80 °C. In patients with more than 4 weeks of chronic diarrhea, the stool was examined for detection of microbial agents. In patients without chronic diarrhea, the stool samples of all patients were examined for viruses of *Norovirus*, *Rotavirus*, *Cytomegalovirus*, and *Enterovirus* by PCR technique.

Polymerase chain reaction (PCR)

The PCR method was performed in a total volume of 25µL. Master Mix used included: Distilled water 12.4 µL, PCR buffer(10X) 5 µL, MgCl₂ 0.6 µL, dNTP (10mM) 0.4, each primer 0.5 µL, Tag polymerase enzyme 1 µL, and DNA sample 1 µL. The PCR steps include primary denaturation of 95 °C for 5 min, a denaturation of 95 °C for 45 Sec, an annealing of 58 °C for 45 Sec, an expansion stage of 72 °C for 45 Sec (40 cycles), and the final stage was 72 °C for 5 min. Samples were electrophoresed on a 1% agarose gel and a 100-bp molecular marker was used. Results were analyzed using gel documentation.

Endoscopy and colonoscopy

Patients who passed various diagnostic examinations and no specific outcome was achieved, as well as the patients who were positive for *Cytomegalovirus* underwent endoscopy and colonoscopy.

Statistical analysis

Comparison between quantitative variables was performed by t-test or in case of abnormal distribution it was performed by

Mann-Whitney U test. The comparison between qualitative variables was also carried out by Chi-square or Fisher's test. Correlation between quantitative variables was analyzed using Pearson coefficient and Spearman tests. All statistical tests were analyzed using SPSS statistical software version 21 (Chicago, IL, USA). A P-value of 0.05 was considered statistically significant.

Results

Fifty-four PID patients completed the questionnaire and entered the study, 48% were females and 52% were males. The average age of males was 26 and females were 28.5. Among these patients, based on the PAGID and ESID, the immunodeficiency and its type were confirmed and the most common category of disease was antibody-deficiency that constituted for 89% of the cases and after that, the phagocytosis efficiency was 3.5%. CVID is the most common disease that constituted about 80% of cases. 22 patients, before the diagnosis of immunodeficiency, had chronic diarrhea. Thirteen patients had diarrhea at the time of entering the study and 9 patients were in the normal state. Among patients who had chronic diarrhea, 2 patients were XLA, 1 patient SCID, 1 patient CMCC and 18 patients with CVID. In all patients, CVID deficiency is the most common type of immunodeficiency that causes diarrhea. In this study, about 85% of CVID patients had chronic diarrhea and after that, XLA about 10%.

In 42 patients, no viruses were found and in 12 patients, viral stool tests were positive. *Norovirus* was the most common virus that detected based on molecular analysis on the stool by PCR. It was positive in 4 patients (7.4%) and in another patient, *Norovirus* was with *Cytomegalovirus*. *Cytomegalovirus* was positive in 3 (5.6%) patients and it was associated with *Norovirus* and *Enterovirus* in 2 patients (Figure 1). Of the 12 patients with the virus in the stool, 8 cases (66.6%) had chronic diarrhea and 4 cases (33.3%) did not have chronic diarrhea during the test. The *Norovirus* and *Cytomegalovirus* were seen only in cases with chronic diarrhea. In all

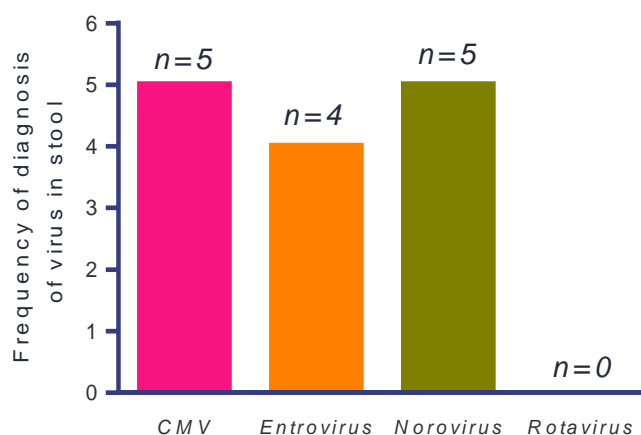


Figure 1: Frequency of virus in stools of the PID patients.

patients with chronic diarrhea (28 patients), in 9 patients (32.1%) the virus was positive in the stool while in the group that never had diarrhea (26 patients), only in 3 patients (11.5%) virus became positive which is significantly associated (P value<0.0001) with diarrhea in patients (Table 1).

		Diagnosis of the virus in the stool						Total (%)	P value
		Negative	Positive						
			CMV	Enterovirus	Norovirus	CMV+ Enterovirus	CMV+ Norovirus		
Chronic diarrhea	Yes (28)	19	3	2	3	0	1	9 (32)	<0.0001
	No (26)	23	0	1	1	1	0	3 (13)	
Total	54	42	3	3	4	1	1	12 (22.2)	

Table 1: Diagnosis of the virus in stool based on the presence of chronic diarrhea.

There was no significant relationship between the number of defecation chronic diarrhea excretion and virus detection in the stool so that the presence of the virus in the stool wasn't significantly associated with those who did not have diarrhea and who had diarrhea with different frequencies. In 10 patients who did colonoscopy and endoscopy, 3 patients had *Cytomegalovirus* that was treated.

Discussion

One of the most complex issues in PID is the study of chronic diarrhea and it involves various parts of the gastrointestinal tract [16]. The referral of patients with severe and chronic diarrhea, along with a disease of immunodeficiency to the clinic led to the algorithmic design that patients are evaluated based on these criteria. CVID is the most common immunodeficiency associated with chronic diarrhea and recurrent infections which have been confirmed in the previous studies [2, 17]. Almost 50 % of CVID patients display a variety of gastrointestinal complaints ranging from dyspepsia to chronic diarrhea [18]. Some immunodeficient patients are susceptible to chronic, often fatal infections with viral pathogens and other viruses of relatively low virulence [19, 20].

Viruses are pathogens that usually do not cause chronic illness in the normal people but in immunocompromised patients, they can cause chronic diarrhea due to various reasons. *Noroviruses* are the most common cause of gastroenteritis in all age groups and viral shedding may both be prolonged in children, older individuals, and immunocompromised patients. *Norovirus* was the main cause of chronic diarrhea in our study. *Norovirus* was found in 4 patients and there was in a patient with *Cytomegalovirus* which was identified in the stool by PCR technique. There were also *Cytomegalovirus* in 3 patients and was associated with *Norovirus* and *Enterovirus* in other 2 patients. Endoscopy and colonoscopy were performed in all patients with *Cytomegalovirus*. In the present study, endoscopy and colonoscopy were performed for 5 patients due to detection of *Cytomegalovirus* in stool PCR and 5 patients due to prolonged chronic diarrhea and negative for all of the assessments. *Cytomegalovirus* in colonoscopy results was only positive for 3 patients that these patients also improved with intravenous Ganciclovir therapy. In 9 of the 22 patients who had chronic diarrhea before the diagnosis of immunodeficiency, diarrhea was significantly interrupted upon increasing the IgG levels.

Viral-associated diarrhea due to *Norovirus*, *Rotavirus*, *Cytomegalovirus* and *enterovirus* is common in immunocompromised patients. Frange et al. evaluated enteric pathogens and *Cytomegalovirus*, *Salmonella*, *Norovirus*, *Shigella*, *Campylobacter*, *Clostridium*, *Microsporidium*, *Cryptosporidium*, and *Norovirus* were reported as the major pathogens in immunocompromised patients. They also studied the prevalence and clinical consequences of *Norovirus* infection in hospitalized children with PIDs. Fecal samples from 62 children were systematically screened for these viruses. *Norovirus* was the most pathogen found in both combined and humoral immunocompromised children. They reported that *Norovirus* was associated with gastrointestinal symptoms and viremia in 54.5% and 25% of cases, respectively [21]. In our study, humoral immunodeficiencies were also the most common immunodeficiency associated with chronic diarrhea and the CVID prevalence was also about 80%. A large Italian study was designed by Sullivan to the natural history of X-linked agammaglobulinemia (XLA). They reported that the prevalence of diarrhea in XLA was 13% [22]. In the present study, the prevalence of XLA was about 10%. In the study of Ghosh et al. about viral-associated diarrhea in immunocompromised and cancer patients in 97 cases, *Norovirus* 49 (59%), *Rotavirus* 34 (74%), and *Adenovirus* 14(78%) were identified [23]. The causes of diarrhea among solid organ transplant (SOT) were studied by Lee et al. that *Norovirus* was positive in 35%. Of patients with diarrhea without *Norovirus*, *Clostridium* (25%) and *Cytomegalovirus* (12%) were the most commonly identified infectious associated with diarrhea. *Norovirus* was a common cause of diarrhea and is responsible for a very prolonged course of chronic diarrhea [24]. The major limitation of this study was small number of immunodeficient patients. A larger sample population is needed. It is also recommended that this study is performed in contribution to other diagnostic studies and further microbial agents such as bacteria and fungi should be considered.

Conclusion

We found *Norovirus*, *Cytomegalovirus*, and *Enterovirus* in the stools of PID patients with chronic diarrhea; therefore, appropriate treatment is applicable to reduce the long-term complications of these patients. However, further investigations are needed to elucidate the virological status of PID patients.