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Original Articles

Gastrointestinal manifestations of Iranian patients with LRBA deficiency

Javad Tafaroji [1*], Fereshte Salami[2], Seyed Alireza Mahdaviani [3], Afshin Shirkani [4], Zahra Chavoshzadeh[5]

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Abstract

Background: Lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency is a rare genetic primary immunodeficiency (PID) disease caused by mutation in the *LRBA* gene. The most important symptoms in patients include autoimmunity, recurrent infections, hypogammaglobulinemia, and enteropathy.

Methods: A total of 19 LRBA patients were enrolled in this longitudinal study. All recorded data for clinical presentation, demographic information, laboratory and gastrointestinal findings were collected.

Results: In this study, 11 females and 8 males (from 16 unrelated families) with LRBA deficiency were evaluated. The most common gastrointestinal symptoms were gastroenteritis, chronic or bloody diarrhea with abdominal pain, vomiting, anorexia, and FTT. The most important pathologic finding was colitis that was seen in 4 patients. Gastritis, esophagitis, gastroesophageal reflux disease, celiac-like disease, and normal upper endoscopy were documented equally in 2 patients. Also seen was enteritis in 3, proctitis, ileitis, and cryptitis in 1, and villous atrophy in 3 of the LRBA patients.

Conclusion: A variety of gastrointestinal conditions may be the most frequent complications in patients with LRBA deficiency.

Keywords LRBA deficiency, primary immunodeficiency, enteropathy, autoimmunity

*** Corresponding author:** Javad Tafaroji
dr.tafaroji@gmail.com

1. Department of Pediatrics, Qom University of Medical Sciences, Qom, Iran

2. Department of Immunology, Yazd University of Medical Sciences, Yazd, Iran

3. Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

4. Allergy and Clinical Immunology Department, Bushehr University of Medical Science, School of Medicine, Bushehr, Iran

5. Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

LRBA (Lipopolysaccharide-responsive beige-like anchor protein) deficiency is a rare genetic primary immune deficiency disease (PID) resulting from biallelic loss-of-function mutations in the LRBA

gene. Immunological abnormalities in patients are decreased IgG antibody production, impairment of specific antibody response, increased apoptosis in B lymphocytes, and defective activation and proliferation of T-cells. In the majority of patients, low B-cell subset counts, especially in switched memory B cells and plasmablasts, have been reported (1, 2). LRBA deficiency has a wide spectrum of clinical phenotypes and manifestations (3-5), including immune deficiency, lymphoproliferation, autoimmunity, and gastrointestinal complications (1, 6).

The most common gastrointestinal symptoms in LRBA deficiency are reported to be chronic and intractable diarrhea (7), chronic active gastritis, active colitis (6, 7), inflammatory bowel disease (IBD) (3, 8), gastric carcinoma and malignant melanoma (9), malabsorption, and failure to thrive (FTT) (10). Recent reports of LRBA deficiency cohorts proposed that enteropathy is a predominant gastrointestinal complication in these patients with a frequency rate ranging from 62% - 76.5% [2, 7, 8].

Until now, there has been no report on gastrointestinal manifestations in patients with LRBA deficiency; therefore, the current study evaluated gastrointestinal manifestations in Iranian patients with LRBA deficiency.

Patients and methods

All 19 LRBA-deficient patients enrolled in this longitudinal study were registered in the Iranian Registry of Primary Immunodeficiency (11). The inclusion criterion was a diagnosis of LRBA deficiency, primarily diagnosed according to standard criteria (12). All patients had a homozygous mutation in the LRBA gene and were diagnosed between March 2013 and October 2017. Any LRBA patient or patient's parents who did not want to participate in the study were excluded. After securing approval for the study from the Ethics Committee of Tehran University of Medical Sciences, informed consent was obtained from parents and patients. Patient records, clinical symptoms, molecular, laboratory, and pathologic data as well as gastrointestinal manifestations and symptoms of the patients were evaluated. Moreover, either an endoscopic or a colonoscopy procedure was performed, and biopsy samples were sent for pathologic study.

Immunological evaluation

Serum immunoglobulin levels and specific antibody responses to tetanus, diphtheria toxoids, and pneumococcal polysaccharide vaccines as well as complete blood counts were measured in all patients according to standard laboratory methods (13, 14). Immunologic evaluations of B- and T- cell subsets (CD markers) and regulatory T cells were done by flow cytometry. Autoantibody and immunoglobulin levels were also evaluated.

Statistical analysis

Values were presented as frequency (number and percentage), mean ± standard deviation (SD), and median (interquartile range, IQR), as appropriate. The Fisher's exact and chi-square tests were used for 2 × 2 comparisons of categorical variables, and the Mann-Whitney U test was used to compare numerical variables. The Shapiro-Wilk test was used

to check the assumption of normality for a variable, and the parametric or nonparametric test was done according to the normality assumption. Statistical analyses were performed using the SPSS software package, version 22 (SPSS Inc., Chicago, IL, USA). A *p*-value <0.05 was considered statistically significant.

Results

Demographic data

This longitudinal study evaluated 19 patients (11 females and 8 males from 16 unrelated families) from Iran with LRBA deficiency. Patients were followed for a median of 13 years per patient (range 1.3 to 33 years). At the time of the study, the median (IQR) age of patients was 15 (6-25) years (**Table 1**). At the time of analysis, 13 patients (68.4%) were alive and 6 patients (31.6%) had died, mostly due to pneumonia, respiratory failure, or gastrointestinal bleeding. All patients were born to consanguineous parents (100%).

Table 1. General data of patients with LRBA deficiency

ID	Sex	Dx	Age (y)	AOO (y)	AOD (y)	A/D	YOF	Zygosity	CDNA mutation	Amino acid changes	CADD score**	Reference	Type of autoimmunity
P1	F	CVID	6	0.0	5	A	5.7	Homozygous	C.1383_1384insAAAGTTAACGTTAGCAGATAGAAGGAAATGATAAA	P.S462LfsX7	32.0	(17)	-
P2	F	CVID	30	2	12	A	28	Homozygous	C.C6607T	P. R2214X	35.5	(17)	-
P3	M	CSD	15	2	4	D	13	Homozygous	C.G175T	P. E59X	37.0	(17)	ITP/AIHA
P4	M	CSD	6	0.6	4	A	5.4	Homozygous	C.544C>T	P. R182X	38.0	(17)	IDDM
P5	F	CVID	13	0.5	6	A	12.4	Homozygous	C.5623delA	P. I1875SfsX14	35.0	(17)	JRA
P6	F	Normal Ig	1.8	0.5	0.6	D	1.3	Homozygous	Large deletion (Exon41)	-	-	(17)	ITP
P7	M	CVID	13	0.5	4	A	12.5	Homozygous	Large deletion (Exon41)	-	-	(17)	ITP/AIHA
P8	F	CVID	22	3	11	D	19	Homozygous	C. 4729+2dupT	-	7.5	(17)	AIHA/ITP/AIT
P9	F	Normal Ig	19	5	10	A	14	Homozygous	C. 4729+2dupT	-	7.5	(17)	ITP/AIHA/Neutropenia
P10	M	CVID	27	2	10	A	25	Homozygous	C.C4814G	P.S1605X	34.5	(17)	-
P11	M	Normal Ig	35	2	34	A	33	Homozygous	C.C4814G	P.S1605X	34.5	(17)	-
P12	F	CVID	21	1	21	A	20	Homozygous	C.C4814G	P.S1605X	34.5	(17)	AIHA/ITP/MS

P13	M	CVID	16	2	8	A	15	Homozygous	C.C544T	P.R182X	38.0	(17)	JIA
P14	M	CVID	19	2	7	D	16	Homozygous	C.G175T	P.E59X	37.0	(17)	ITP/AIHA
P15	F	CVID	11	3	5	D	6	Homozygous	C. 1014+1G>A	-	26.5	(17)	JRA
P16	F	CVID	19	2	17	D	17	Homozygous	Large deletion (Exon 1-2 deletion)	-	-	(17)	Myasthenia Gravis
P17	M	CVID	12	2	7	A	10	Homozygous	C.743_744insAAGA	P.D248EfsX	36.0	(17)	AIHA/ITP
P18	F	CVID	4	1	3	A	2	Homozygous	C.C4814G	-	-	New patient	-
P19	F	CVID	2	1	1	A	2	Homozygous	NM-001199282: exon 29, C.4638delc	-	-	New patient	ITP/AIHA

Dx, Diagnosis at the time of mutation analysis; AOO, age of onset; AOD, age of diagnosis; A/D, alive or dead; YOF, years of follow-up; IHA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura; JRA, juvenile rheumatoid arthritis; MS, multiple sclerosis; IDDM, insulin-dependent diabetes mellitus; AIT, autoimmune thrombocytopenia. All patients had consanguineous parents

Clinical manifestations

The most common presentations of immunodeficiency at the onset of disease were respiratory tract infection (n=7; 36.8%), chronic diarrhea (n=4; 21.1%), autoimmunity (n=3; 15.8%), failure to thrive (FTT) (n=1; 5.3%), allergy and asthma (n=2; 10.5%), and fever (n=2; 10.5%). The median (IQR) age at the primary onset of symptoms was 2.0 (0.55-2.0) years, and the age at the time of diagnosis was 7.0 (4.5-11.5) years (patient data illustrated in **Table 1**). During the follow-up of patients, the main LRBA phenotypes were chronic diarrhea and enteropathy, infection, autoimmunity and lymphoproliferative disease (**Figure 1**).

All patients (19 of 19) had a history of infectious complications. Infection manifestations were bacterial and viral pneumonia in 14 (73.7%), otitis media in 13 (68.4), sinusitis in 13 (68.4%), meningitis in 3 (15.8%), brain abscess in 1 (5.3%), septicemia in 2 (10.5%), and oral candidiasis in 4 (21.1%) patients. Patients 10 and 14 (P10 and P14) had Giardia lamblia, and P13 had nematode parasite infection with Trichostrongylidae.

Pulmonary infection was the most common respiratory infection by a median (IQR) of 0.33 (0.02-0.51) episodes per year. P3, P4, and P17 had Cytomegalovirus pneumonia, and P7 was infected with Epstein-Barr virus and had Pseudomonas septicemia.

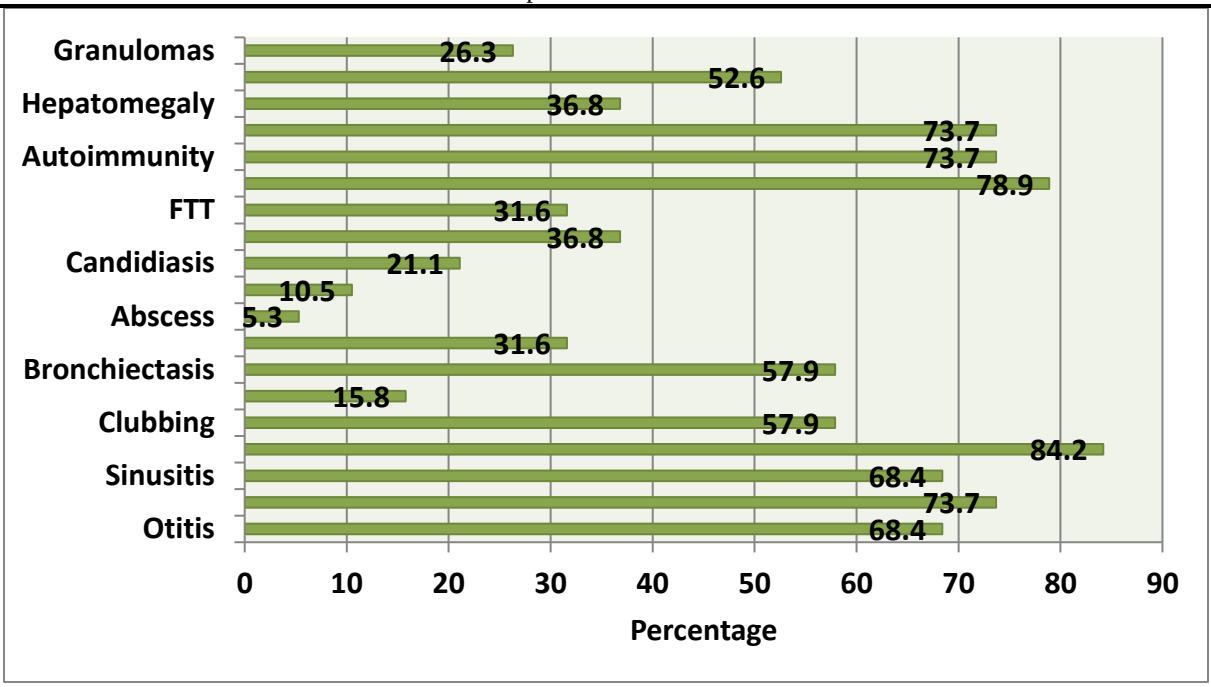
Cholecystitis was reported in P7, P12 had acute hepatitis, and allergic problems were seen in P6, P8, P14, and P16. P10 was infected with Helicobacter pylori, and disseminated varicella infection, oral thrush, brain abscess, Pseudomonas aeruginosa, Pneumocystis jirovecii and CMV were seen in P17. Disseminated leishmaniasis and hypertriglyceridemia was also reported in P19.

In the current study, 14 (82.3%) patients had a history of lymphoproliferative disorders, including, hepatomegaly, splenomegaly, lymphadenopathy, and granuloma. Non-caseating granuloma was

reported in five patients (26.3%), and three patients presented with granulomatous-lymphocytic interstitial lung disease. All three of them had a history of lung infection with the CMV virus. Multiple hepatic, splenic, adrenal, and pulmonary granuloma were seen in P13.

A variety of autoimmune, endocrine, neurological, hematological, and rheumatologic disorders were reported in 14 (73.7%) patients with LRBA deficiency (**Table 1**). Atopic disorders including allergic dermatitis, food allergies, urticaria, asthma, and insect sting allergies were also documented in six of the 19 (31.6%) patients evaluated. Bronchiectasis was reported in 11 (57.9%) and clubbing in 11 (51.7%) of the LRBA patients. P5 had septic arthritis.

Figure 1. Clinical manifestations of LRBA-deficient patients



Gastrointestinal manifestations

The most common gastrointestinal symptoms in LRBA patients were gastroenteritis (84.2%), chronic or bloody diarrhea with abdominal pain, vomiting, anorexia (84.2%), and FTT (31.6%).

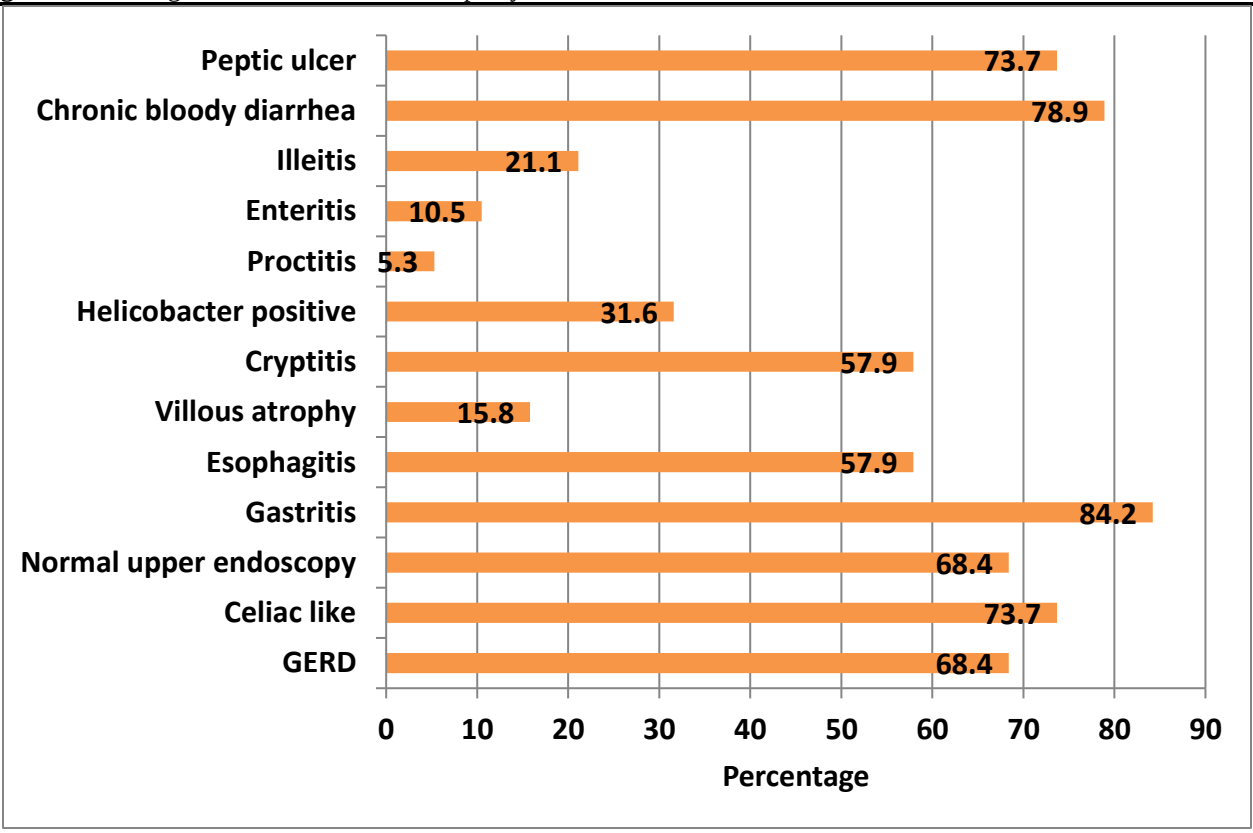
Chronic diarrhea was found in 16 patients (84.2%) patients, 11 of whom (57.8%) manifested three or more episodes of diarrhea during follow-up. Gastrointestinal symptoms were reported in 15 (78.9%) patients, and enteropathy proven by biopsy was seen in nine patients (47.3%). Chronic active gastritis, chronic non-crypt destructive colitis, active colitis, villous atrophy, and intestinal inflammation were the most common and important findings in the patients' pathology reports. Six patients had FTT, while five of these six patients had a history of enteropathy. Endoscopy or colonoscopy was performed on 15 (78.9) LRBA patients with gastrointestinal symptoms (based on patient

symptoms and signs). The most common enteropathic conditions were inflammatory bowel disease (IBD) seen in 8 (47.3%) patients and celiac-like disease in 2 (12.5%) patients (**Figure 2**).

The most important pathologic finding in biopsies was colitis seen in four patients (25%). Gastritis, esophagitis, GERD, celiac–like disease, and normal upper endoscopy were documented equally in 2 (12.5%) patients. Enteritis was seen in 3 (17.6%), proctitis, ileitis, and cryptitis in 1 (6.3%), and villous atrophy in 3 (18.8%) LRBA patients. H. pylori was found in one of the patients after an endoscopic procedure (6.3%), and peptic ulcer was seen in another patient. Chronic bloody diarrhea was also seen in one patient (6.3%) (**Figure 2**).

Twelve patients had an overlap of autoimmune and enteropathy disease. It was found that age at onset of enteropathy was less than that of autoimmunity (2.5 [1.0-5.0] vs. 3.0 [2.0-6.0] years); however, the differences were not significant. According to the assessments, none of the patients with GERD had autoimmune cytopenia, but 50% of non-GERD patients had an autoimmune cytopenia. The incidence of autoimmune cytopenia in those with celiac-like disease was 50% compared to 42.9% in those who did not have celiac-like disease.

Figure 2. Pathologic manifestations of enteropathy in LRBA disease



The prevalence of autoimmune cytopenia in those with enteritis disease was 66.7% compared to 42.9% in those who did not have enteritis. In patients with villous atrophy, 2 patients (66.7%)

had autoimmune cytopenia, but in patients who did not have villous atrophy, 5 patients (38.5%) had autoimmunity. Moreover, autoimmune cytopenia was seen in 25% of patients with colitis and in 50% of those without it.

The prevalence of rheumatologic disorders in patients with gastritis was 0%, but 14.3% of patients who did not have gastritis had a rheumatologic disorder (p=0.350). None of the patients with GERD, celiac-like disease, or enteritis had rheumatologic disorders, but 21.4% of patients without these diseases had rheumatologic disorders. The incidence of rheumatologic disorders in patients with villous atrophy was 0% compared to 23.1% in those who did not have villous atrophy. Rheumatologic disorders were seen in 25% of patients with colitis, but in only 16.7% of patients who did not.

Discussion

LRBA deficiency has several clinical phenotypes, the most of which are chronic diarrhea, autoimmune disorders, hypogammaglobulinemia, respiratory tract infection (15), organomegaly, or combinations of these phenotypes (2, 4, 6, 16). Bal et al. described six symptomatic patients with LRBA deficiency, including autoimmunity (6/6), organomegaly (6/6), and chronic diarrhea (5/6). In other study, Azizi et al. reported pneumonia (76.5%), lymphoproliferative disorders (82.3%), and enteropathy (76.5%) as the main clinical phenotypes of LRBA deficiency (17, 18). Gámez-Díaz et al. described 13 LRBA patients with enteropathy (61.9%) (2), while in the current study 15 patients (78.9%) had a history of enteropathy and 8 patients (42.1%) had a diagnosis of IBD. This represents a higher percentage of enteropathy in the current study due to the focus on LRBA patients with gastrointestinal complications. Therefore, LRBA patients may be associated only with enteropathy symptoms without infectious complications, and gastroenterologists, immunologists, and rheumatologists should be aware of the clinical symptoms of this disease.

Before the discovery of LRBA gene mutations, most LRBA patients were diagnosed with CVID. In a study by Gámez-Díaz et al. (2), 41% of patients with LRBA had a previous diagnosis of CVID. Lopez-Herrera et al. reported on five patients who had a childhood-onset CVID diagnosis (6). In the current study, however, 14 (73.7%) LRBA patients had a primary diagnosis of CVID. Therefore, most CVID patients with autoimmune complications and enteropathy may have defects in the LRBA gene.

The current study is the first pathological study of gastrointestinal involvement in LRBA patients. Biopsies were performed in 15 (78.9%) patients with persistent gastrointestinal symptoms. The most enteropathy-type complications were celiac-like disease and IBD. The pathologic findings of gastrointestinal biopsies included colitis, GERD, celiac-like disease, gastritis, esophagitis, villous atrophy, cryptitis, proctitis, enteritis, ileitis, and peptic ulcer. One patient

tested positive for Helicobacter pylori, and 2 patients had a normal upper gastrointestinal endoscopy. Burns et al. and Alangari et al., in two different reports, showed that duodenal villous atrophy, lymphocytic colitis or Crohn's-like disease occur in LRBA patients (15, 19, 20). This data indicates that LRBA can affect any part of the gastrointestinal tract, from the mouth to the anus. Usually, in pathologic studies of biopsies, the infiltration of inflammatory cells in the mucosal membrane of the digestive system are critical findings for a diagnosis of inflammatory complication in the gastrointestinal tract. It was reported that in the histology of jejunum mucosa in coeliac and IBD in CVID, LRBA, and XLA patients, the lack of plasma cells is typical and most striking, while an increased number of intra-epithelial lymphocytes, granulomas, and crypt distortions should be observed. Because of these differences, inflammatory gastrointestinal diseases in CVID and LRBA patients are named "coeliac-like", "Crohn's-like", "sprue-like", and so on. This should be considered in the pathologic examination of a gastrointestinal biopsy by an expert pathologist when a question of LRBA disease exists.

Conflicts of interest The authors declare that they have no conflicts of interest.

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