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Original Article

Genetic Evaluation of Patients Suspected of Immunodeficiency Referred to the Immunodeficiency Clinic of Akbar Hospital in Mashhad

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Abstract

Background: The purpose of this study was genetic evaluation of patients suspected of immunodeficiency, without a definitive diagnosis, referred to the Immunodeficiency Clinic of Akbar Hospital in Mashhad in 2021-2022.

Methods: In this study, patients suspected of immunodeficiency, without a definitive diagnosis, referred to an immunodeficiency clinic were included A complete clinical and paraclinical examination has been done by expert specialists and clinical geneticists. Blood samples were taken for genetic analysis using the Exome Sequencing technique followed by comprehensive bioinformatics analysis. Parents and healthy offspring were assessed for the candidate gene variants.

Results: In this study, 185 patients were included; 58.56% of them were male; The average age of the participants was 9.28±5.40 years, and consanguineous marriage of parents was observed in 79.8 % of cases. Pneumonia with 33.51% was the most common clinical manifestation in patients with suspected immunodeficiency. In total, 41.14% of patients suffered from combined immunodeficiency, 26 .86% of them had defects of phagocyte number, function, or both; and 24% had predominantly antibody deficiencies. Hyper IgE syndrome was detected in 16% of patients, SCID and CGD each in 14.86% of patients, CVID in 12% of patients, and LAD in 7.43% of them. In 37.04% of the identified genes, there was a discrepancy between clinical and genetic diagnosis in patients.

Conclusion: The most common clinical manifestation of patients suspected of primary immunodeficiency is pneumonia; therefore, patients who suffer from recurrent respiratory infections should be checked for genetic immunodeficiency. In this study, most patients were in the groups of immunodeficiencies affecting multiple cell types, defects of phagocyte number, function, or both; and predominantly antibody deficiencies, respectively. The most common diseases diagnosed were: Hyper IgE syndrome, SCID and CGD, CVID, and LAD.

Keywords: Genetic; Immunodeficiency; Clinical Diagnosis; Molecular Analysis; PID.

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Introduction

Primary immunodeficiencies (PIDs), also called interchangeably as inborn errors of immunity (IEI) are a heterogeneous group of congenital disorders of the immune system (1). These disorders are different from secondary immunodeficiencies, which are caused by a variety of more than 400 serious diseases (2). PID cases are prone to different bacteria, viral, and even fungal infections (3). The condition is believed to be rare; however, there may be a suspicious increasing pattern during the last decades. It is reported that PID affects 1 out of every 8500 to 100000 people in different parts of the world. However, it is believed that the prevalence should be much higher, as there are asymptomatic cases, too (1).

A genetic screening, using 329 predefined genes, should have a prevalence of 1 in 1349 people in the USA (4). Aghamohammadi et al. also conducted a study in Iran. They reported 731 PID cases, in which antibody deficiencies constituted 32.3% of the patients, as the most predominant disorder (5). Ahanchian et al. proposed that patients with recurrent infections may be suspected as immunodeficient cases. They reported a PID rate of 26.8% of the assessed cases (6). In this regard, genetic assessment plays a crucial role in those, who are suspected of PIDs (7). Usually, insufficient data and late diagnosis of PID patients result in mismanagement of the cases. Therefore, genetic assessment is important in timely diagnosis, confirmation of suspected condition, selected therapy, and good management of the patients (8). An-time diagnosis of PID, using genetic testing can further improve the survival of these cases. Moreover, genetic assessment can help even targeted gene therapy (9). Lastly, the parents need to avoid the birth of PID children in future pregnancies. Still, the gene bank of the PID-related genes should be further completed (10). This study aims to evaluate the genetic profile of patients suspected of immunodeficiency, without definitive diagnosis.

Method and Materials

Study design and sample

In this cross-sectional study, patients referred to Akbar Hospital's immunology clinic with suspicion of PID, whose definitive diagnosis was not determined despite all laboratory evaluations, were included in the study. To make a definitive diagnosis and carry out appropriate treatment, referred them to the genetic clinic, and blood samples were taken from them for genetic analysis.

Probands were selected from affected individuals in families referred to the genetic clinic for genetic counseling due to a primary diagnosis of immune deficiency. 5 ml of peripheral blood with EDTA was obtained from patients and other available family members. DNA was extracted using standards salting out protocol. The proband's DNA samples were sent to Macrogen Company (Korea) for whole-exome sequencing (WES) 100X.

Genetic testing and bioinformatic analysis

After data analysis, the polymerase chain reaction (PCR) technique was performed using specific primers to confirm the candidate genetic variants, followed by Sanger sequencing. We interpreted and classified sequence variants through the Human Gene Mutation Database (HGMD) and ClinVar. In the case of a novel variant, it was classified as pathogenic, likely pathogenic, uncertain significance, likely benign, or benign according to the American College of Medical Genetics and Genomics (ACMG, 2015) guideline.

Ethics

All the patients or their legal guardians were provided with written informed consent. The patients were free to continue the study. Moreover, the data of the patients were anonymized and coded in order to be kept secret. All the steps of the study were in accordance with Helsinki's declaration. The ethics committee of Mashhad University of Medical Sciences confirmed the study protocol (Ethics code: IR.MUMS.MEDICAL.REC.1400.219

Result

In total, 185 suspected PID cases, including 106 male patients (58.56%) and 79 (41.44%) were enrolled in the study. The mean age of the included cases was 9.28±5.40 years old, ranging from 1 year to 26 years old. Unfortunately, 33 cases (18.3%) died during the time of study and their data were extracted from their documents. In total, 11 cases (0.05%) had parents with familial marriage, in 96 patients (71.6%) the parents were second-degree relatives, and in 27 cases (20.1%) there was no familial marriage. **Figure 1** shows the main clinical presentation of the patients. The most common presentation was pneumonia which was present in 62 cases (33.51%).

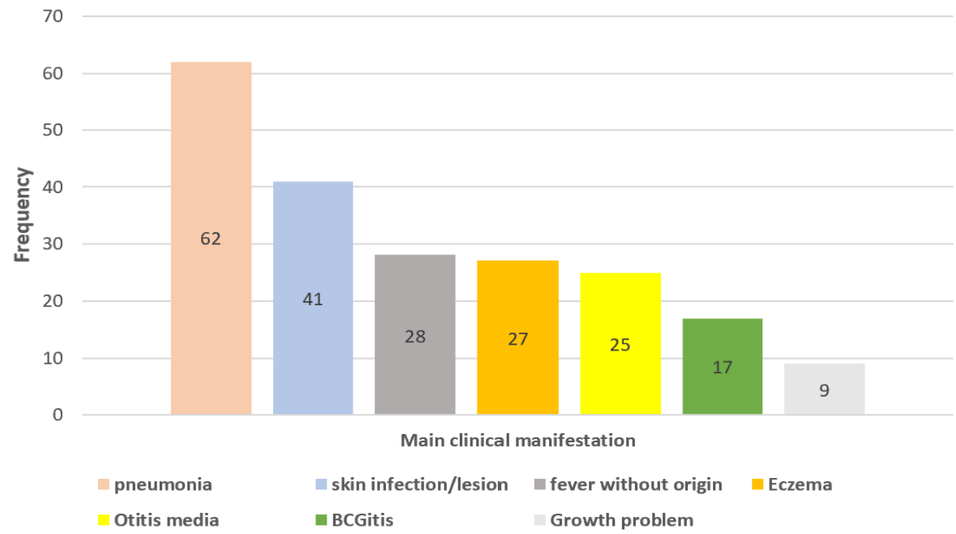

Figure 1. The bar plot of the frequency of the patient's presentation

Table 1 shows the clinical diagnosis of the studied cases. As it is evident, the final diagnosis is classified into different categories including combined immunodeficiency, primary humoral immunodeficiency, phagocyte dysfunction, innate immunity deficiency, immune system malfunction, autoimmunity, and bone marrow failure. The most common category was combined immunodeficiency (41.41%) and the list commons were autoimmunity and bone marrow failure (0.57%). Exome sequencing results are demonstrated in **Table 2.**

Figure 2 also shows the distribution of different clinical manifestations according to the clinical diagnosis. After genetic assessment, a total of 30 genetic defects were identified; however, three of them were not included in the table provided by the expert committee of the International Union of Immunological Societies (IUIS). Inconsistency between primary clinical diagnosis and genetic diagnosis of the patient was seen in 10 cases (37.04%). The details of 27 cases are demonstrated in **Table 3**. The results of the genetic assessment of the three patients, which was not provided in IUIS were as follows:

Patient 1 had a CVID diagnosis and mutation in the SHPK and the UNC80 genes.
Patient 2 had a CVID diagnosis and mutation in the MMA gene.
Patient 3 had an SCID diagnosis and a mutation in the TLR7 gene.

Table 1. The frequency and percent of clinical diagnosis

Categories	Clinical diagnosis	Frequency (%)	Total percent
Combined immunodeficiency	CID	3 (1.71)	41.41
	SCID	26 (14.86)	
	Congenital thrombocytopenia	1 (0.57)	
	WAS	2 (1.14)	
	ARPC1B Deficiency	3 (1.71)	
	AT	9 (5.14)	
	Hyper IgE syndrome	28 (16.00)	
Primary humoral immunodeficiency	Antibody dysfunction	5 (2.86)	24.00
	CVID	21 (12.00)	
	Agammaglobulinemia	3 (1.71)	
	XLA	10 (5.71)	
	Hyper IgM syndrome	2 (1.14)	
	Hypogammaglobulinemia	1 (0.57)	
Phagocytes dysfunction	Congenital neutropenia	8 (4.57)	26.86
	CGD	26 (14.68)	
	LAD	13 (7.43)	
	CHS	1 (0.57)	
Innate immunity deficiency	MSMD	10 (5.71)	5.71
Immune system malfunction	ALPS	2 (1.14)	1.71
T cell dysfunction disorder	Di-George syndrome	1 (0.57)	1 (0.57)
Autoimmunity	TRAPS	1 (0.57)	0.57
Bone marrow failure	DKC	1 (0.57)	0.57

CID, combined immunodeficiency; SCID, severe combined immunodeficiency; WAS, Wiskott Aldrich syndrome; AT, ataxia telangiectasia; CVID, common variable immunodeficiency; XLA, X-linked agammaglobulinemia; CGD, chronic granulomatous disease; LAD, leukocyte adhesion deficiency; MSMD, Mendelian susceptibility to mycobacterial disease; ALPS, autoimmune lymphoproliferative syndrome; CHS, Chediak Higashi syndrome; TRAPS, tumor necrosis factor receptor-associated periodic syndrome; DKC, Dyskeratosis congenital

Table 2. Genome sequencing results

Genes	Frequency	Percent
WES	5	9.80
ATM	4	7.84
STAT3	3	5.88
IFNGR1	3	5.88
WAS	3	5.88
ARPC1B	3	5.88
ADA	2	3.92
DOCK8	2	3.92
LRBA	2	3.92
RAG1/RAG2	2	3.92
DNMT3B	2	3.92
CASP8	1	1.96

Genes	Frequency	Percent
STAT2	1	1.96
CD79A	1	1.96
CD21	1	1.96
NHEJ1	1	1.96
ITGB2/TTGB2	1	1.96
UNG	1	1.96
ELANE	1	1.96
JAK3	1	1.96
PIK3CD	1	1.96
MALT1	1	1.96
IL12RB2	1	1.96
HAX1	1	1.96
RFX5	1	1.96
CR2 (complement receptor 2)	1	1.96
TP53	1	1.96
TLR7	1	1.96
MMA	1	1.96
SHPK/UNC80	1	1.96
DEL 22	1	1.96

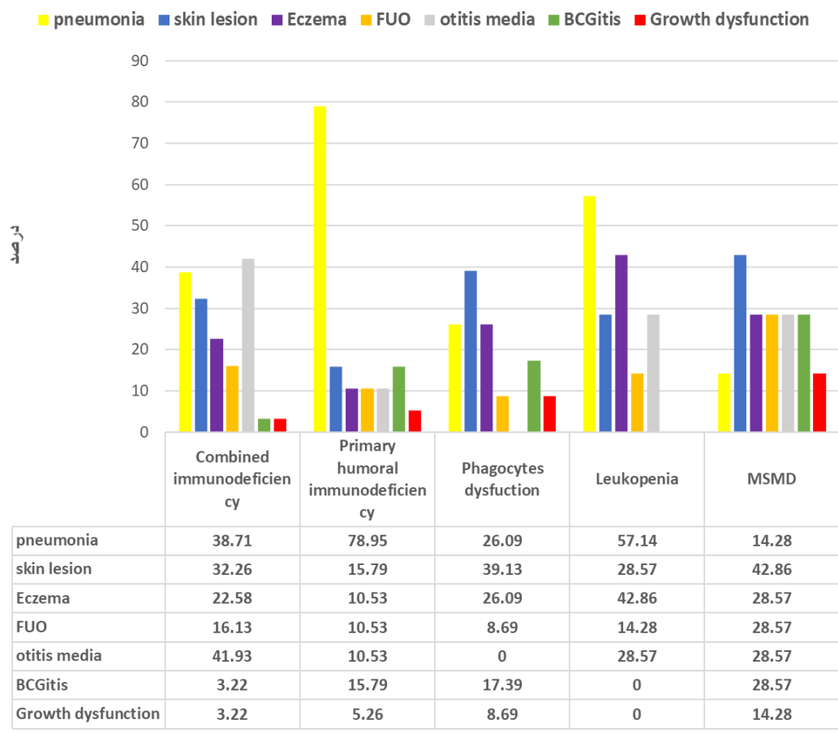

Figure 2. The distribution of different clinical manifestations according to the clinical diagnosis.

Table 3. Clinical diagnosis, genetic diagnosis, defective gene, and the main group of primary immunodeficiency

	Clinical diagnosis	Genetic diagnosis	Defected gene	main group of primary immunodeficiency
1	SCID	ADA deficiency	ADA	Combined
2	ARPC1B deficiency	ARPC1B deficiency	ARPC1B	Combined (syndromic)
3	AT	AT	ATM	Combined (syndromic)
4	LAD	ALPS-caspase8	CASP8	Immune malfunction
5	Hypogammaglobulinemia	IgA deficiency	CD79A	Humoral (antibody)
6	Antibody dysfunction	CD21 deficiency	CD21	Humoral (antibody)
7	Antibody dysfunction	ICF1	DNMT3B	Combined (syndromic)
8	Hyper IgE	DOCK8 deficiency	DOCK8	Combined
9	Neutropenia	Elastase deficiency	ELANE	Phagocyte dysfunction
10	Neutropenia	Kostmann disease	HAX1	Phagocyte dysfunction
11	MSMD	IFN-g receptor1 deficiency	IFNGR1	Innate immunity
12	MSMD	IL-12Rb2 deficiency	IL12RB2	Innate immunity
13	LAD1	LAD1	ITGB2	Phagocyte dysfunction
14	SCID	JAK3 deficiency	JAK3	Combined
15	Hyper IgE	LRBA deficiency	LRBA	Immune malfunction
16	CVID	LRBA deficiency	LRBA	Immune malfunction
17	Antibody dysfunction	MALT1 deficiency	MALT1	Combined
18	XLA	Cernunnos/XLF deficiency	NHEJ1	Combined
19	PIK3CD deficiency	PIK3CD deficiency	PIK3CD	Humoral (antibody)
20	CVID	PIK3CD deficiency	PIK3CD	Humoral (antibody)
21	SCID	RAG1 deficiency	RAG1	Combined
22	MHC II deficiency	MHC class II deficiency, Group C	RFX5	Combined
23	Hyper IgE	STAT2 deficiency	STAT2	Innate immunity
24	Hyper IgE	AD-HIES Job syndrome	STAT3	Combined
25	AT	BMFS5	TP53 (C.467G>A)	Bone marrow failure
26	Hyper IgM	UNG deficiency	UNG	Humoral (antibody)
27	WAS	WAS	WAS	Combined (syndromic)

Discussion

Genetic assessment of PID cases is a very important factor. Molecular analysis of these cases can reach in better understanding of the disease's nature, susceptibility to different infectious organisms, and possible accompanying non-infectious diseases, as a part of the syndrome. Furthermore, the genetic result plays a crucial role in patients' treatment including targeted gene therapy. Therefore, studies and registries all around the world try to present the genetic results of PID patients. Our study was one of these genetic assessments. The gene analysis of our cases concluded that 30 patients with 3 cases revealed de novo mutations in SHPK, UNC80, MMA, and TLR7 genes. The most common group of primary immunodeficiency according to the genetic analysis was combined immunodeficiency, which was found in 12 out of 27 cases (44.44%), followed by humoral immunodeficiency (5 cases; 18.51%), phagocyte dysfunction (3 cases; 11.11%), innate immunodeficiency (3 cases; 11.11%), Immune malfunction (3 cases; 11.11%), and bone marrow failure (1 case; 3.70%).

The fourth update of the Iranian National Registry of Primary Immunodeficiencies proposed the presumed prevalence of primary immunodeficiencies to be more than 1/600. The putative causative genetic defect was identified in 33.1% of the patients in their study. The rate was 16.21% in our study. They proposed that the most common category of PIDs was predominantly antibody deficiencies, followed by autoinflammatory disorders, immunodeficiencies affecting cellular and humoral immunity, and combined immunodeficiencies with associated or syndromic features (11). Antibody deficiencies were also the top listed disorder in our study. It seems that the result of this study should be incorporated into national surveys with this regard for further completion. Moreover, our study was focused on the pediatric population, and the differences between our study and the national registry are due to this fact. A 6-year genetic survey in Switzerland proposed the minimal prevalence of PID in this country to be 4.2 patients per 100,000 inhabitants. Predominantly antibody disorders were the most common diseases observed (62%), followed by phagocytic disorders (9%). However, they reported that predominantly antibody disorders were more common in adults than in children. Similar to our study, the commonly found PID in children was combined immunodeficiency in the Marschall et al. study (12). Another study conducted in Germany, for 5 years, showed that the most common PIDs in this country were common variable immunodeficiency (30%) and unclassified antibody deficiency (11%). In our study, combined immunodeficiency and humoral immunodeficiency were the most common conditions (13). Herz et al. conducted another study in a community with high consanguinity. They reported that 70% of all assessed cases had a genetic finding regarding. They also reported that immunodeficiencies affecting cellular and humoral immunity (35.2%) and combined immunodeficiencies (24%) were the most common disorders in their population (14).

Our study provided valuable data on the genetic findings of the patients with PID. However, these data are much more valuable in a national registry and can be useful in this regard. Therefore, the limited number of cases in our study can be addressed as a shortcoming. Genetic testing is vital in those, who are highly suspected of immunodeficiency. This test results in prompt diagnosis of the disease and further prevention of involved cases of birth.

Conclusion

The positivity rate of genetic disorders found in PID-suspected cases in our center was 16.21 percent. We found three cases with new mutations in SHPK, UNC80, MMA, and TLR7 genes. In our pediatric population, the most commonly seen group of primary immunodeficiency was combined immunodeficiency, which was found in 44.44 percent of positive cases. The findings of this study should be completed with further national studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

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