CASE REPORT
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MHC Class II Deficiency with Normal CD4$^+$ T Cell Counts: A Case Report

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ABSTRACT

Major histocompatibility complex (MHC) class II deficiency is a rare primary immunodeficiency disorder (PID) with less than 200 cases worldwide. Here, we report an 8 month–old girl with MHC class II deficiency with a novel homozygous mutation in *RFXANK* gene *(NM_001278728: exon 5: c.495G>A: p.Trp165*)* and normal CD4$^+$ T cell counts, diagnosed by whole exome sequencing (WES) and negative *HLA–DR* proteins on peripheral blood mononuclear cell (PBMC) in flow cytometry. She was referred with pneumonia, prolonged fever, resistance to antibiotics (ceftriaxone, clindamycin, and vancomycin), and low serum immunoglobulin (IG) levels, while natural killer (NK), B, and T cells were normal. She received intra-venous immune-globulin (IVIG) replacement, broad spectrum antibiotics, and anti-fungal treatments. The presented case report is interesting not only because of the rarity of the PID but also due to normal CD4$^+$ T cell counts. According to our experience, we suggest that physicians consider MHC class II deficiency in families with consanguineous marriages, even with normal CD4$^+$ T cell counts. At the first, the diagnosis of the disease could be successfully perform using WES, and finally, treatment with hematopoietic stem cell transplantation can save the patients' lives.

Keywords: Genetic diseases; Human; Immunologic deficiency syndromes; Inborn; Molecular sequence data RFXANK protein

INTRODUCTION

Major histocompatibility complex (MHC) class II deficiency or bare lymphocyte syndrome (BLS) type II is an extremely rare combined immunodeficiency disorder, and so far less than 200 cases in the world, the most frequent in the Mediterranean, have been reported.[1] The main clinical characteristic of MHC class II deficiency is severe and recurrent viral, bacterial, and fungal infections and most patients are often identified with some specific symptoms; such as common laboratory findings include partial or complete absence of human leucocyte antigen (HLA)–DR,–DP

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and–DQ gene expression, and absence of cellular and humoral immune response, especially CD4$^+$ cells, which is the underlying cause for susceptibility of the affected patients to infections.[2]

MHC class II deficiency is a congenital autosomal recessive primary immunodeficiency disorder (PID), which it often involves children of consanguineous couples, and it can be the main reason for the higher prevalence of this disease in the Middle East and North Africa.[3] MHC class II deficiency has a poor prognosis and was associated with a reduction in survival rate. Therefore, well-timed diagnosis and treatment are crucial for these patients.[4] Molecular studies around the world have identified various mutations in different groups involving with this disease, such as *(CIITA), RFXANK, RFX5*, and *RFXAP* mutations, as mutations in complementation groups A–D, respectively,[5] among which *RFXANK* genes have been identified as the most severe and fatal mutation, requiring hematopoietic stem cell transplantation (HSCT) as the only therapeutic approach.[6] However, according to the geographic region, the dominant mutation is different in each population; for example, *752delG26* is the most prevalent in North Africa.[7,8] Few studies have been conducted on the genetic outcomes of Iranian patients[9,10] due to the lack of attention of the researcher.[11] Therefore, studies like this study focusing on the genetic principles of Iranian patients can help identify the most prevalent genetic mutations in Iran.

In addition, it is known that MHC class II molecules are expressed on cells that are considered as antigen presenting cells (APCs), such as macrophages, monocytes, dendritic cells, and B cells. Therefore, the low CD4$^+$ cell count is considered as one of the key cues of this disease,[12] while we present a case of MHC class II deficiency with normal CD4$^+$ cell counts and a rare mutation. Due to the rarity of this disease, reporting cases with special characteristics can help diagnose these patients more appropriately and the onset of a timely treatment may reduce the mortality rate.

CASE PRESENTATION

A 6–month–old girl was admitted to the centre for some symptoms, such as pneumonia, prolonged fever, and broad-spectrum antibiotic resistance, like ceftriaxone (Ceftriaxone-exir 1G vial), clindamycin (Clindamycin-Soha Charbagh 300 mg cap), and vancomycin (Vancomycin-exir 500 mg vial). She received BCG vaccine at birth and did not show any complications. At 6 months of age, the patient's weight, head circumference, and height was 6200g [between percentile 5th -10th], 41 cm [between percentile 10th-25th], 67 cm [75 percentile], respectively. So taken together, the physiological situation was near normal. The patient had no history of hospitalization before six months of age, and was admitted to hospital located in Khorramabad, Iran for the first time at this age with pneumonia due to some symptoms, including dyspnea, cyanosis of the organs and mouth as well as respiratory distress, and was therefore received several antibiotics, such as vancomycin, clindamycin, ceftriaxone, and oral azithromycin.

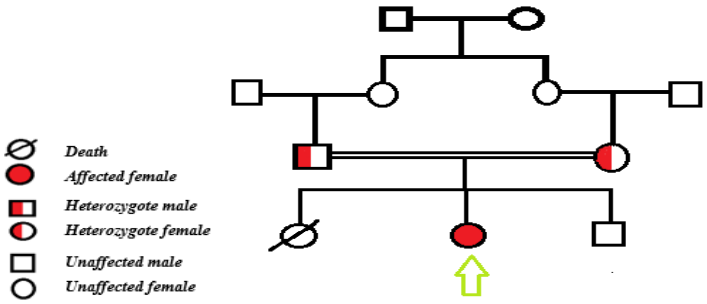

Figure 1. The pedigree of a patient with MHC class II deficiency with normal CD4+ T cell counts, which shows the history of her family in terms of inheritance of the mutation of the RFXANK gene. The analysis of this pedigree demonstrates that parents have a heterozygote form of *RFXANK* gene mutation, and during their genetic pattern transfer to their children, they lost their first child without clear evidence, and the second child appears the disease in a homozygous recessive state of *RFXANK* gene mutation, while the third child is completely healthy.

Table 1. The results of two rounds of the laboratory test of a patient with MHC class II deficiency with normal CD4+ T cell counts

Test	Result (1)	Result (2)	Unit	Normal Range
W.B.C	16.1	7.1	/ml	4000-10000
Neutrophils	63%	58%		
Lymphocyte	37%*	40%		
Eosinophil	_	2%		
Platelet	348	224	10^3/uL	130-400
CRP	+++	Negative		
HIV Abs	Negative	Negative		
HIV PCR	Negative	Negative		
HLA-DR	Negative	Negative		

Ab, antibodyy; HLA-DR, Human Leukocyte antigen_DR

*The patient has transient leukopenia during a limited period of hospitalization, which associated with sepsis.

The patient was referred to Abuzar Hospital in Ahvaz for more inspection. The patient had cyanosis at the time of admission to the center, although this situation was resolved with 97 percent oxygen. A few days earlier, signs of diarrhea and vomiting were improving, although there was no metabolic acidosis. Therefore, she was treated with clindamycin, vancomycin (90 mg every 6 hours), and meropenem (180 mg every 8 hours) for twenty days. However, intravenous cotrimoxazol was replaced after 48 hours due to lack of response to clindamycin. In addition, due to a family history of immunodeficiency and some symptoms was suggestive of Combine Immuno-Deficiency, such as resistant to infection, fever, diarrhea, and vomiting, as well as failure to respond to antibiotic therapy, 3 gr of intra-venous immune-globulin (IVIG) in every month was prescribed for her.

The parents had a consanguineous marriage (the cousin- the daughter of aunt type) with no positive history of PIDs, while they had a child expired with no definite diagnosis at the age of five and the patient's sister had received IVIG during his lifetime due to the possible diagnosis of hypo-gamma-globulinemia.

Clinical examination revealed ill, double-sided crackles coarse, very low oxygen saturation, open anterior fontanel with tip finger size, purpura and petechiae lesions in the lower jaw area and around the neck, occurrence of respiratory dystrophy and diarrhea following recent vaccination.

Furthermore, the results of the laboratory test showed a transient leukopenia, which can be a sign of sepsis. The results of the patient's laboratory findings and flow cytometry data are shown in Table 1 and 2. However, cell count results in flow-cytometric test showed that ratio of the T CD4+ to T CD8+ was lower than the normal range, although B cell and natural killer (NK) cell count in this patient's peripheral blood were normal. Besides, the serum level of IgA and IgM have decreased (IgA <10 mg/dl; NV [20-100 mg/dl]) (IgM<10 mg/dl; NV [19-146 mg/dl]), and also the amount of serum IgG sharply decreased (IgG: 160 mg/dl; NV [453-916 mg/dl]).

Table 2. The results of two rounds of flow cytometry and CBC tests in a patient with MHC class II deficiency with normal CD4+ T cell counts

CD Markers	Result (1)	Result (2)	Unit	Normal Range
CD3	45.3 (2101)	60 (2783)	% of Lymph (Absolut Count)	30-78 (1391-3618)
CD4	28.0 (1299)	33 (1531)	% of Lymph (Absolut Count)	22-58 (1021-2691)
CD8	20.4 (946)	23(1066)	% of Lymph (Absolut Count)	10-37 (464-1716)
CD4/CD8	1.4	1.4	_	1-4
CD19	27.4 (1271)	32 (1484)	% of Lymph (Absolut Count)	9-38 (418-1763)
CD56	18.3 (848)	7 (324)	% of Lymph (Absolut Count)	3-15 (139-694)
CD16	20.5 (950)	?	% of Lymph (Absolut Count)	5-19 (232-880)
W.B.C	7.1	5.0	/ml	4000-10000
Neutrophils	58%	47%	% of total	
Lymphocyte	40%	47%	% of total	
Eosinophil	2%	3%	% of total	

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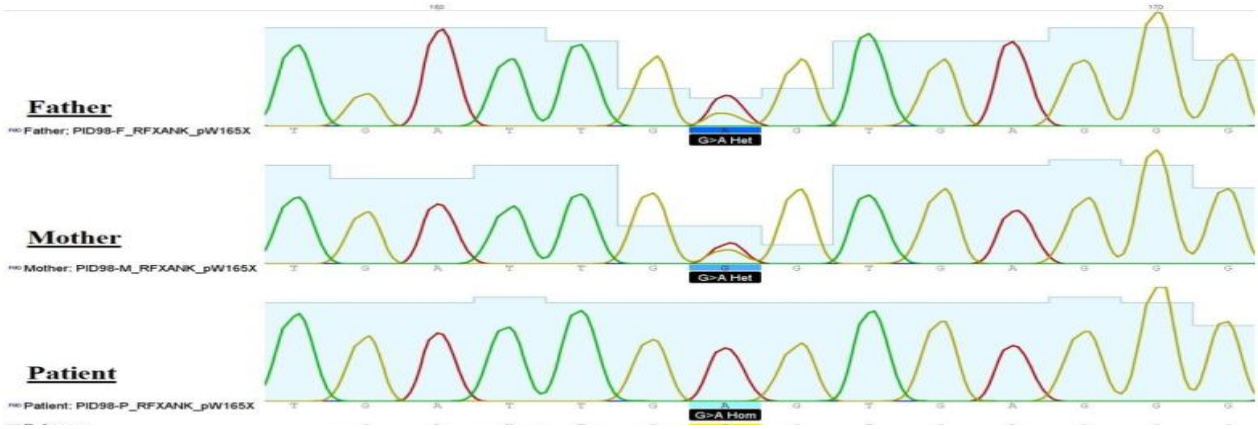

Figure 2. A novel homozygous mutation in the *RFXANK* gene *(NM_001278728: exon 5: c.495G>A: p.Trp165*)* was found that leads to a stop–gain mutation which can lead to protein dysfunction. Sanger sequencing confirmed that the patient is homozygous and the parents (father and mother) were both heterozygous carriers for this mutation. ("[*]" is the symbol of the termination of the sequence)

The patient was suspected to have combine immunodeficiency, therefore, IVIG replacement, broad–spectrum antibiotics, and antifungal treatments were started for the patient. Due to clinical symptoms including fever and skin rash, antibiotic-resistant infections, and most importantly, history of death in the patient's family due to immunodeficiency and considering the results of the cell count and the low ratio of T CD4$^+$ to T CD8$^+$, as well as the reduction in all classes of antibodies, the patient is diagnosed as combine immunodeficiency. Despite all clinical symptoms indicating combine immunodeficiency, the results of the genetic test carried out on the patient's sample showed the presence of the mutation in the RFXANK gene, which indicates BLS.

The patient underwent Whole Exome Sequencing (WES) for a definite diagnosis. MHC class II expression was investigated looking for *HLA–DR* proteins on the surface of the peripheral blood mononuclear cell (PBMC) by flow cytometry. A novel homozygous mutation in *RFXANK* gene *(NM_001278728: exon 5: c.495G>A: p.Trp165*)* was found that leads to a stop–gain mutation which can ID cause protein dysfunction (Figure 2). Sanger sequencing confirmed that the patient is homozygous and the parents (father and mother) were both heterozygous carriers for this mutation. *HLA–DR* expression was negative, confirmed MHC II deficiency in this patient. WES sequence in the present study was performed by Illumine high throughput DNA sequencing technology and the values were compared to standard references and parental WES and mutations were interpreted by the board–certified laboratory clinicians in accordance with clinical findings. Finally, after a 23-day course of supportive care and HSCT, complete patient consciousness and healing of vital signs were achieved. Also, cyanosis and respiratory dystrophy were completely eliminated.

Usually in patients with MHC-deficiency, symptoms such as chronic diarrhea, skin rash, and failure to thrive and underweight, but interestingly, the patient presented doesn't show any of them, and the growth curve and patient's weight were near normal (at the age of 2.5 (after about 2 years); his height, weight, and the head circumference were 89.5cm [50th percentile], 11.950 kg [25th percentile] and 47.0 cm [25th percentile], respectively). In addition, although the function of the CD4$^+$ T cells was defective [as revealed in Table 3], the count of these cells was normal. Taken together, the presence of this novel mutation causes the count of the CD4$^+$ T cells to be normal despite the lack of HLA-DR on the antigen presenting leukocytes, and thus the symptoms 0f the disease is milder compared to other mutations known in this locus of the gene. The objectives of the study were explained to the parents of the child and written informed consent was signed by the father for using the child's information in this study. No additional costs were imposed on the parents for the sake of the study. All principles of Helsinki's declaration for reporting human studies were met throughout the study steps.

Table 3. The result of the lymphocyte transformation test (LTT) test in a patient with MHC class II deficiency with normal CD4+ T cell counts to evaluate the functional status of the patient's T cell

Test	Patient	Control	Normal Range
Phytohaemagglutinin (PHA) test	3.2	3.5	≥ 3
Bacillus Calmette–Guérin (BCG) test	2.1	3.0	≥ 2.5
Candidiasis test	1.5	3.2	≥ 2.5

DISCUSSION

In this study, we reported a case with a rare disease, with less than 200 patients reported worldwide, while the case presented had a novel mutation in *RFXANK* gene *(NM_001278728: exon 5: c.495G>A: p.Trp165*)* and Among the clinical findings, resistance to antibiotics and among laboratory findings, low serum immunoglobulin (IG) levels suspected the physician to PID, in addition to the family history of the patient (consanguineous marriage and a sick sister suspected to die of a sort of PID). Therefore, WES was requested for the patient that revealed MHC class II deficiency and flow cytometry revealed negative *HLA–DR* proteins on the peripheral blood mononuclear cell (PBMC), however; his parents were both heterozygous carriers.

The presented case had a novel genetic mutation in the *RFXANK* gene, while studies have reported different genetic results. For example, in North Africa, *752delG26*,[7,8] and in Tunisia, *25_338del26* is reported as the most prevalent mutation,[2] while very few studies have been reported on the genetic evaluation of these patients in Iran. In another study, Farrokhi and colleagues reported a 5-year-old Iranian boy with homozygous mutation in *RFXANK* gene on *19p12* as a substitution of *G* with A in *TGG* codon for *W188* that resulted in a premature stop codon creation in exon 3 and caused recurrent fever, diarrhoea, failure to thrive, upper respiratory tract infection, and oral lesions since four months of age.[11] The reported mutation in this study is different from our presented case but was similarly in the *RFXANK* gene. As this mutation is considered serious and lethal and requires non-conservative treatments like HSCT.[6] Given these new gene mutations, this suggestion is proposed that[11] should pay more attention to this gene in Iran.

In addition, although the overall prevalence of MHC class II deficiency is not considerable around the world, this illness is one of the most important diseases in countries with high prevalence of consanguineous marriages, such as the Middle East and North Africa.[3] Notably, 22 of 25 Maghrebian[12] 8 out of 11 Algerian,[8] and 8 out of 10 Moroccan patients[7] had a history of consanguinity that indicates the great role of consanguinity in MHC class II deficiency. Another finding of the presented patient was normal counts of B cells and NK cells, albeit low levels of $CD4^+$ T cells.[1,4,8] In a case series of 11 Algerian patients, all patients lacked HLA DR surface expression on monocytes, while 2 patients had normal $CD4^+$ T cells,[2] which is consistent with the case presented in this study. Therefore, although the molecular explanation suggests the involvement of MHC class II in $CD4^+$ T cells,[13] the diagnosis should not be made based on $CD4^+$ T cells count in patients suspected of MHC class II deficiency.

In Iran, the high prevalence of illness can be attributed to consanguineous parents, as epidemiologic reports state that nearly 40% of couples with different ethnicities have consanguinity,[14] which most probably, lies under the historical background of Iranian culture.[15] Therefore, it is essential that policy makers pay greater attention to including a premarital genetic study for all couples with consanguinity, and including assessment of PID in the genetic study. According to our experience, WES can be a reliable diagnostic tool for the identification of heterozygote mutations.

In the present case, treatment with IVIG replacement, broad–spectrum antibiotics, and antifungal treatments was initiated for the patient for preventing further complications of the patients' infections, while the effective treatment used for the patient was HSCT, identified as an effective treatment for PIDs with long-term survival rate.[16] This case introduced in this study, interestingly responded to the treatment, completely. It has been identified that response to HSCT may be limited in patients with MHC class II deficiency[17] and depend on various

factors, such as HLA–identically of transplant recipients, reliable donor stem cell engraftment, and immune reconstitution.[18] In the presented case, one of the factors that can lead to undesirable results in treatment can be related to the normal $CD4^+$ T cell counts, as $CD4^+$ leukopenia are suggested as the factor for successful HSCT.[19]

The resent case report can help physicians, researchers, and policy makers on a wider view on the cases with MHC class II deficiency. According to the very few genetic studies on patients with MHC class II deficiency in Iran, and unknown frequency of the most common symptoms, clinical and laboratory findings, and the responsible mutations, it is suggested that further studies evaluate and report such cases, due to the high prevalence of consanguinity in Iran suggest the higher prevalence of MHC class II deficiency in Iran compared with other countries in the world.

The interesting points of the presented case included a novel mutation in the *RFXANK* gene *(NM_001278728: exon 5: c.495G>A: p.Trp165*)* and a bizarre laboratory finding (normal $CD4^+$ T cell counts). According to our experience, we suggest that physicians consider MHC class II deficiency in families with consanguineous marriages, even with normal $CD4^+$ T cell counts, as early diagnosis of the disease with WES and treatment with HSCT can save the patients' lives.

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