Brief Report

A novel frame shift mutation in *STIM1* gene causing primary immunodeficiency

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SUMMARY　Immunodeficiency 10 is an autosomal recessive disorder presenting with iris hypoplasia, muscular hypotonia and nonprogressive myopathy, recurrent bacterial infections, autoimmune hemolytic anemia, hypohidrosis and nail dysplasia caused by the mutation of stromal interaction molecule 1 gene (*STIM1*). Herein, we present a new case of *STIM1* mediated immunodeficiency, carrying a novel frameshift mutation. Our patient presented with nephrotic syndrome, hypotonia, myopathy, recurrent bacterial infections, thrombocytopenia and autoimmune hemolytic anemia. She is now 23 months old and is on steroid, cyclosporine and monthly IVIG. She has had no recent significant infections and is receiving rehabilitation therapy to improve her motor skills. Rare genetic syndromes should be suspected in patients of consanguineous parents, who present with a set of different manifestations. Gathering all the patient's manifestations together and looking them as one disease should be encouraged.

Keywords　genetics, immunology, recurrent infections, pediatrics

1. Introduction

Immunodeficiency 10 is an autosomal recessive disorder presenting with iris hypoplasia, muscular hypotonia and nonprogressive myopathy, recurrent bacterial infections, autoimmune hemolytic anemia, hypohidrosis and nail dysplasia (*1*). The genetic base of this syndrome is due to the mutation of stromal interaction molecule 1 gene (*STIM1*) located on chromosome 11p15.4. The *STIM1* gene provides instructions for encoding STIM1 protein, which is involved in controlling the entry of positively charged calcium ions into the cells through calcium-release activated calcium (CRAC) channels when levels of the ions are low (*2*). These proteins have been showed to be localized to the endoplasmic reticulum and the plasma membrane, serving as Ca sensors and, also, mediators for Ca influx (*3*).

Here we report a case of immunodeficiency 10 presented with nonprogressive myopathy and hypotonia, and nephrotic syndrome.

2. Patients and Methods

Our patient is a 2-year-old girl of consanguineous parents. During early infancy, her parents noticed sucking and swallowing discoordination of their daughter. In her monthly pediatrician visits, mild

degrees of delay in motor developmental milestones was observed. At 7 months old, she developed sudden onset fever accompanied with lethargy, poor feeding and generalized edema; work-ups showed nephrotic range proteinuria along with hyperlipidemia and hypoalbuminemia with normal renal function and normal liver enzymes. Kidney biopsy pathology was suggestive of classic features of Focal Segmental glomerulosclerosis, Not Otherwise Specified (NOS) variant. Prednisolone and Enalapril was started for her and, fortunately, due to her good response to the prescribed medicine, proteinuria abated after few months; therefore, steroid dosage was tapered off. One year later when she was 17 months old, generalized petechial and ecchymotic rashes were appeared on her body. Lab data revealed thrombocytopenia, normal hemoglobin (Hb) level and normal white blood cell (WBC) count. Erythrocyte sedimentation rate (ESR) was 47 mm/hr and C-reactive protein (CRP) was 7 mg/dL. Due to previous history of kidney involvement and nephrotic syndrome, lupus serology and complements were checked which were all normal.

She underwent bone marrow aspiration and biopsy which revealed normocellular marrow and Flow cytometric results for Bernard-Soulier syndrome and Glanzmann's thrombasthenia were both negative. Therefore, she was diagnosed as Idiopathic Thrombocytopenic Purpura (ITP) and methylprednisolone was administrated for

her, but no satisfactory clinical response was detected. Few days later, she developed sudden onset hemolytic anemia with positive indirect coombs test ,normal G6PD level, reticulocyte count: 10% and erythropoietin level > 750 IU/L. Considering ITP and hemolytic anemia, possibility of Evans' syndrome was deliberated and due to ineffectiveness of steroid pulse administration, Rituximab was initiated 375mg/m^2 weekly for four consecutive weeks. Fortunately, our patient had good clinical response after the fourth dose of Rituximab, with normal platelet and Hb level; moreover, she did not have proteinuria any more. In Parallel to these manifestations and considering the patient's history and evidences of repeated infections including otitis media and urinary tract infection, consultation with an expert pediatric immunologist was measured and cluster of differentiation (CD) marker flow cytometry, Dihydrorhodamine (DHR) test and immunoglobulin levels were requested. CD flow cytometry demonstrated low CD19, CD20 and CD8 and high CD3 and CD4. However, as the results could be attributed to rituximab, repeating the work-ups later was recommended. DHR was negative and immunoglobulin levels were in normal range. Therefore, steroid dose was tapered gradually.

At 19 months old, she was hospitalized due to coughing, respiratory distress and episodes of lip cyanosis. Chest x-ray revealed bilateral ground glass appearance but no evidence of infectious process was found in her lab data (normal CRP and procalcitonin). Spiral Chest CT scan demonstrated bilateral patchy infiltrations, diffuse ground glass appearance and mosaic pattern in middle lobe and lingula more likely associated with vasculitis. Her desired clinical response to methylprednisolone pulse was also corroborative of vasculitis rather than infections. After 1 week of respiratory care and mechanical ventilation, she was extubated (however, the patient was still oxygen dependent). Bronchoscopy was also done which documented swallowing discoordination by fiberoptic endoscopic evaluation of swallowing (FEES) and malacia of larynx, trachea, and left main stem and revealed mucosal plugging in right main stem and right lower lobe. Microscopic examination of the bronchoalveolar lavage was negative for bacterial and fungal infections. During PICU admission, she was noticed to have mid-dilated pupils, which were both non-reactive to light stimulation. Hence, brain MRI, MR Angiography and MR venography were requested which revealed diffuse microbleeding in different part of both cerebral hemispheres, suggestive of vasculopathy. Later, with ophthalmologist consult, it was specified that the patient has iris hypoplasia (partial aniridia) and non-reactive pupils. As stated earlier, our patient had mild motor skill developmental delay and hypotonia; therefore, electromyography was performed for her, reported mild myopathic process, and found no evidence of peripheral neuropathy.

3. Results and Discussion

Considering her delayed motor skill development, renal involvement, history of thrombocytopenia, recurrent infections and autoimmune disorders including autoimmune hemolytic anemia and vasculitis, something syndromic were suspected; hence, whole exome sequencing (WES) was ordered which revealed a novel homozygous mutation in *STIM1* gene (with 11p15.4 cytogenetic location) and based on our patient clinical picture, "immunodeficiency 10 syndrome" was suggested which was compatible with her clinical manifestations.

Immunodeficiency 10 is a rare genetic disorder presenting with variable features and severity, most commonly recurrent episodes of infections, nonprogressive myopathy and muscular hypotonia (*4,5*). The defective known gene associated with this kind of primary immunodeficiency is *STIM1* which is located on Chromosome 11 and at least three loss-of-function mutations are detected to have a pivotal role in pathogenesis of immunodeficiency 10 (*6,7*). These mutations result in impaired calcium ions influx into cells which lead to defects in gene expression, cell growth and division, and immune dysfunction particularly NK-cell and T-cell inactivation (*8,9*). Immunodeficiency 10 syndrome is of disorders with autosomal recessive heritance with rare allele distribution throughout population (*10*); so, this pattern suggests a common ancestor to be present between parents. All reported cases with this immunodeficiency (Table 1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=66*) had positive parental consanguinity, as our patient had.

Based on the determining role of *STIM1* gene in biologic intracellular and extracellular cascades, its manifestations usually emerge early, in neonacy or infancy (*1*). The clinical manifestations and the extent of immune system dysfunction mainly depends on the type of the mutation that affected the *STIM1* gene. The first three reported patients (based on Table 1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=66*) had complete alteration of *STIM1* gene functions due to homozygous nonsense mutations in the *STIM1* gene (E136X mutation) and therefore, significantly reduced STIM1 protein production, which resulted in severe immunologic features including immunodeficiency, autoimmune disease, and myopathy (*5*). The first two siblings died early in age, one at the age of 18 months from encephalitis and the other at the age of nine years from complications of hematopoietic stem-cell transplantation (HSCT); however, fortunately, the other sibling survived after HSCT and there are no immunological or infectious complications. He does suffer from moderate muscular weakness but he is completely ambulatory and does not need a wheelchair or other support. The fourth reported patient, died at the age of 2 years and four months from severe pulmonary

infection, was shown to have a homozygous loss-of-function point mutation in STIM1 alleles, determined by studying EBV-transformed B cells (EBV-B cells) from the patient (the only material remained from the deceased patient). This point mutation leads to a complete impaired production of STIM1 protein and therefore an impaired store-operated Ca^{2+} entry (SOCE) (*11*). The next two siblings, both, with homozygous R429C point mutation however had residual *STIM1* gene expression, the SOCE was completely depleted leading to pronounced defect in T-cell activation and NK cell function with partial response to viral infections (*12*). One sibling died in 21 months due to suspected sepsis but the other one is alive now at the age of 14-year-old after two times of HSCT (rejected the first graft).

Patients 8 and 9 were reported to have a hypomorphic homozygous missense *STIM1* mutation (mutation c.494C>A in exon 4 resulting in p.165P>Q) which get rise to reduced but residual STIM1 protein production and SOCE which is sufficient for some levels of T-cell proliferation and activation (*10*); therefore, this could explain different immunologic features of these patients comparing to the aforementioned reported cases that these two siblings had no immune cytopenia and no episodes of lymphoproliferation. These two patients would have such a prolonged survival without HSCT. They are both alive now- the boy (patient 8) suffers from severe colitis and the girl (patient 9) have recurrent episodes of skin infection. Likewise, the patient 10 and 11 (cousins) with missense homozygous STIM1 mutation (p.L74P- c.221T>C) was reported to have no permanent immune cytopenia and overt clinical immunodeficiency despite severe SOCE impairment (*13*). Another extraordinary feature seen in these two patients is that in contrast to all aforementioned reported patients, they were not affected with any level of myopathy. In our patient, a novel homozygous frameshift deletion mutation in exon 7 (p. T273fs- c.818_831del) was reported.

Recurrent severe infections are of characteristics of *STIM1*-associated immunodeficiency. Despite bacterial infections such as sepsis and upper respiratory tract infections, these patients due to obvious defective T- and NK-cell function have a pronounced susceptibility to intracellular infections and particularly to viruses (*14*). As demonstrated in Table 1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=66*), viral infections especially Herpesviridae infections (Herpes Simplex Virus, Varicella Zoster Virus, Cytomegalovirus, Epstein Barr virus, and Human Herpes Virus 8) are of pathogens of concern. In 2009, Picard *et al.* reported three patients from one kindred with immunodeficiency 10 syndrome. The older child had caught CMV infection at 1 month of age and two episodes of chickenpox (VZV infection) before 4 year old. The middle child, also, had been reported to contract EBV infection and enteroviral encephalitis (*5*). In another case-report by Minji Byun *et*

al., their patient had been found to have classic Kaposi sarcoma caused by HHV8 infection (*11*). In 2012, Feske *et al.* reported two sisters with *STIM1*-mediated immunodeficiency, which both of whom had suffered recurrent viral pneumonias, recurrent HSV stomatitis, and chronic EBV and CMV viremia (*12*).

Whereas nephrotic syndrome has not been directly included in immunodeficiency 10 entity (*1*), nephrotic syndrome was present in our patient and some of other patients with *STIM1*-mediated immunodeficiency. Nephrotic syndrome was detected in our case after presenting with lymphadenopathy, poor feeding, proteinuria, and edema at 7 month old. In a case-report by Picard *et al.*, one of patients with immunodeficiency 10 syndrome had severe nephrotic syndrome (*5*) and in another one by Stefan Feske *et al.*, one of patients with *ORAI1* and *STIM1*-mediated immunodeficiency had suffered from nephrotic syndrome (*6*). Maybe in future with advance of scientific methods and research, the role of *STIM1* gene in underlying pathways involved in nephrotic syndrome pathogenesis become uncovered. Of another manifestations in primary immunodeficiencies is vasculitis, either in internal organs such as brain (*15*), and lung (*16*) or in external organ, the skin (17). The most common specific vasculitis found in patients with primary immunodeficiency has been reported to be CNS vasculitis (*18*). CNS vasculitis was observed in our patient based on some evidences of diffuse microbleeding in both cerebral hemispheres. In the same way, both lungs especially middle lobe and lingula had been involved by vasculitis based on evidences of patchy infiltrations, diffuse ground glass appearance and mosaic pattern reported in CT scan.

To sum up, it would be of great importance to remind that however, this genetic disorder has its complications, but with several measures, their life quality and life expectancy would be improved. Using steroids would be protective against nephrotic syndrome and autoimmune processes including autoimmune hemolytic anemia, immune thrombocytopenia and vasculitis and since steroids may have long-term adverse sequelae such as neurocognitive impairment, stunting, obesity, cardiac dysfunction and infertility (*19*), steroid sparing agents could be administrated, permitting partial withdrawal of glucocorticoids and reducing glucocorticoids' adverse effects (*20*). Use of HSCT for those with moderately to severely suffered immune system, would be helpful in immune system improvement, preventing fatal infections (*21*). Immunization is also another effective choice that can be measured in patients with primary immunodeficiencies (*22*). Fortunately, their myopathy is nonprogressive (*1*); so, with occupational therapy and physical therapy, their ambulation would improve (*23*). Our patients is now 23 months old and is on steroid and cyclosporine as an steroid sparing agent as she has developed signs of steroid toxicity in appearance. She has had no recent significant infection and is receiving

rehabilitation therapy to improve her motor skills. Reviewing the previous articles on this rare genetic disorder, we initiated treatment with monthly intravenous immunoglobulin (IVIG) for her.

4. Conclusion

Rare genetic syndromes should be suspected in patients of consanguineous parents, who present with a set of different manifestations. Gathering all the patient's manifestations together and looking them as one disease should be encouraged. Such approaches are the key to earlier identification of congenital genetic disorders and will lead to awareness of the underlying problem of the patient instead of symptomatic therapy, and by reviewing the past reported articles in this regard; stronger decisions will be made about the course of the disease, recommended medications and, the patient's future, and prognosis.

Acknowledgements

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