Current Research in Translational Medicine xxx (2018) xxx–xxx

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Brief communication

X-linked hyper-IgM syndrome associated with pulmonary manifestations: A very rare case of functional mutation in CD40L gene in Iran

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ARTICLE INFO

Article history:
Received 3 October 2017
Accepted 7 February 2018
Available online xxx

Keywords:
Hyper IgM syndrome
CD40 ligand
Whole exome sequencing

ABSTRACT

Hyper IgM (HIGM) syndromes are a complex of primary immunodeficiency disorders. A 4-years-old boy with recurrent fever and chills, dyspnea, sort throat for a month was admitted to emergency department. In the current case, whole exome sequencing followed by Sanger sequencing were employed in order to screen probable functional mutations. Molecular analysis revealed a functional mutation across the *CD40L* gene (NM_000074: exon5: c.T464C) resulted in amino acid change p.L155P attributed to X-linked hyper IgM syndrome. The findings of the current study signify the critical role of microbial infection as well as XHIGM screening, particularly in those children cases with respiratory symptoms.

1. Introduction

Hyper IgM (HIGM) syndromes are a complex of primary immunodeficiency disorders that was firstly defined by Burtin and Rosen at the early of 1960 decade [1]. HIGM is resulting from mutations trough a number of genes that lead to immunoglobulin class switch recombination [2]. Patients who carry the mutations, mostly present normal or elevated levels of IgM and suffer from deficiency of IgG, IgA and IgE. Until now, mutation in couple of genes responsible for this disorder has been characterized [3]. According to recent classification, HIGM can be grouped into 8 separate genetically classes. However, damaging mutation in CD40 ligand (CD40L, CD154) gene is known as the most common X-linked HIGM and covers about 65–70% of HIGM cases [4,5].

It seems that most of HIGM patients suffer from X-linked damaging mutation in *CD40L* gene would lead to malfunction of activated T cells [6]. Although some available techniques like flow-cytometry have introduced to identify non-functional CD40L protein [3,7], most of them are not available in diagnostic Labs, particularly in developing countries. Therefore, it seems that the actual number of HIGM cases due to CD40L deficiency is underestimated.

HIGM patients naturally are more prone to upper and lower respiratory tract infection not only resulting from primary infection due to *Pneumocystis jiroveci*, but also secondary local or systematic opportunistic infections by fungi, bacteria and parasites [1,6]. There are strong evidences, showing increased rate of broad spectrum of malignancy (such as hepatocellular carcinoma, GI tract neoplasms and neuroendocrine carcinoma), autoimmune disorders and intermittent/persistent neutropenia among HIGM patients [6]. In the current study, mutation of *CD40L* gene related to HIGM was reported from a 4-years-old boy who suffered from pulmonary complications. To our knowledge, this is the first case of mutation of *CD40L* gene responsible for XHIGM in Iran.

Abbreviations: HIGM, Hyper IgM; CD40L, CD40 ligand; WES, whole exome sequencing; PAP, pulmonary alveolar proteinosis; PJP, *P. jiroveci* pneumonia.

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https://doi.org/10.1016/j.retram.2018.02.001
2452-3186/

2. Case report

2.1. Clinical features

A 4-years-old boy with recurrent fever and chills, dyspnea, sort throat for a month with history of antibiotic therapy was admitted to the emergency department. He had history of recurrent sino-respiratory symptoms and cold, approximately every month with no sufficient responses to antibiotic therapy. The child had watery discharges from his eyes and nose, cough and history of allergic symptoms particularly in autumn. He has had dysphonia and anxiety during recent two years. Diagnostic test for *Mycobacterium tuberculosis* was prescribed and done that the result was negative.

There was no history of gastrointestinal disorders despite dysphagia and slight losing weight during the last 2 months. The pregnancy of his mother was normal and he was born after a cesarean. All the routine vaccines were received without any notable complication. Antibody response to tetanus, diphtheria and pertussis vaccines was evaluated that the levels of them were low. His parents had far family marriage and his mother's brother was dead because of pneumonia when he was 1-year-old. After physical examinations, pharynx was slightly erythemic with tumescent tonsils but without exudate. Auscultation of lungs showed no moist rales, despite rough breath sound. Abdominal examination and percussion revealed light distention. There was no organomegaly and mass.

Laboratory findings were: white blood cell (WBC) 2.01 mm $\times$ 1000 mm with cell differentiation segment: 5.5%, lymphocyte: 57.7% and monocyte: 35.8%; red blood cell (RBC): 4.65 mm $\times$ 10^6 mm; Platelet: 526 mm $\times$ 1000 mm; Hb: 9.8 g/dL; Hct: 28.9 mm; MCV: 62.2 fL; MCH: 21.1 Pgm; MCHC: 33.9; RDW-CV: 15.7; PDW: 8.9 fL; MPV: 8.5 fL; ESR: 80 mm/h; RBC morphology was microcytic 1+ and hypochromic 1+. C-reactive protein was evaluated using serological test and showed 12 mg/L. Complement C3 and C4 were 2.68 g/L and 0.20 g/L, respectively. Serum level of IgA was 0.3 g/L, IgG >1.8 g/L, IgE <0.1 IU/L, while the level of IgM was significantly high and more than 4.6 g/L (Table 1). In this case neutropenia was observed that was episodic; therefore, one dose GCSF was described. The IgA and IgG to *Toxoplasma gondii* were evaluated using ELISA and were negative. PPD test for tuberculosis (TB) was negative. In order to assess the infectivity with *P. jiroveci*, sputum was collected. Then, DNA extraction was performed and the presence of specific fragment for *P. jiroveci* was tested using PCR that the result was negative. Furthermore, In this case, presence of CD40L had to demonstrate using flow cytometry that because of some limitations in order to provide laboratory kits, performing this test was not possible. However, some other CD markers were assessed (Table 2).

Chest radiography was applied and showed several reticulo-nodular infiltrations in lower 5.5 mm zone of each lobes and left hilum (Fig. 1).

The current study was approved by the Ethic Committee of Iran University of Medical Sciences. An informed consent was filled by parents of the patient for further molecular tests. For this purpose, peripheral blood was collected (EDTA-coagulated) from the child. The genomic DNA was extracted, and mono whole exome

Table 1
Hematological ranges of the patient in comparison with normal ranges.

Parameters	Ranges	Reference ranges
Red blood cell count (10^{12}/L)	4.65	4.5–6.3
Hemoglobin (gm/dL)	9.8	14–18
Haematocrit (%)	38.9	39–52
MCV (fl)	62.2	77–97
MCHC (gm/dL)	33.9	32–36
MCH (Pgm)	21.1	26–32
White blood cell count (10^9/L)	2.01	4–10
Segment (%)	5.5	40–80
Lymphocytes (%)	57.7	20–40
Monocytes (%)	36.8	2–10
Platelet (10^9/L)	526	140–440
C reactive protein (mg/L)	12	0–6
Serum level of immunoglobulin[a]		
IgA (g/L)	0.3	0.27–1.95
IgG (g/L)	>1.8	5.04–14.64
IgE (IU/L)	<0.1	0.02–3.07
IgM (g/L)	>4.6	0.24–2.10
Complement component (g/L)		
C3	2.68	0.9–1.8
C4	0.20	0.1–0.4

Abbreviations: MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin.
[a] Reference range of immunoglobulins are mentioned based on normal range for age 4–6 years.

Table 2
Flowcytometric values of lymphocyte's CD markers of the patient.

CD markers	Values (%)	Reference values (%)
CD3	68	56–75
CD4	45	28–47
CD8	22	16–30
CD16	10	4.5–30
CD19	13	3.5–15.5
CD20	13.5	3.5–17
CD22	12	3.5–15.5
CD45 Ro	25	9–26
CD56	10.5	4.5–30

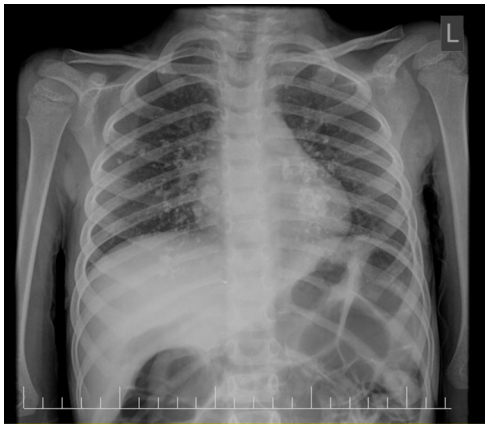

Fig. 1. Chest X-ray showed reticulonodular infiltrations in the lobes and left hilum of the patient.

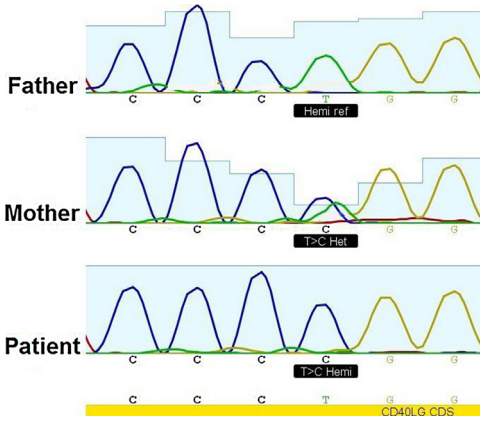

Fig. 2. Polymorphism analysis of patient and his mother and father. Father had normal sequence while molecular analysis showed that patient's mother carries heterozygote mutation (T and C).

Please cite this article in press as: Torabizadeh M, et al. X-linked hyper-IgM syndrome associated with pulmonary manifestations: A very rare case of functional mutation in CD40L gene in Iran. Curr Res Transl Med (2018), https://doi.org/10.1016/j.retram.2018.02.001

sequencing (WES) test followed by mutation confirmation using direct Sanger sequencing were carried out (Fig. 2).

Molecular analysis revealed mutation in the *CD40L* gene (NM_000074: exon5: c.T464C) that was led to amino acid change p.L155P, which has been reported as cause of hyper IgM syndrome.

3. Discussion

HIGM is a rare immunity disorder resulting from mutation in the *CD40L* gene. The patients often are known with normal or high level of IgM and low level of other class of immunoglobulins. These patients are more susceptible to opportunistic infections as well as malignancy [8,9]. This is the first study describing a case of HIGM with new mutation in *CD40L* gene in Iran.

Our patient represented low to normal level of IgA, IgG and IgE, in spite of significant elevated level of IgM. Until now, several cases of deficiency in CD 27 $^{+}$ memory B cell producing IgM and IgD in peripheral blood have been described who suffered from HIGM. These classical features were previously reported in HIGM cases from all over the world [10,11]. However, long-term antigen simulation due to malfunction of T cells and abnormal development of B cells was proposed as the main reason of elevation of level of IgM [12].

Susceptibility to opportunistic infections due to malfunction of T cell and abnormal development of memory B cells are considered as the most important concern of physicians [4,13]. Together with lack/reduction of expression of CD40L in the majority cases of HIGM, CD40L malfunction is frequently reported from HIGM patients [3,6,7]. However, almost 20% of patients who suffer from XHIGM die before 25 years of old due to *P. jiroveci* pneumonia (PJP) and liver diseases [1].

In the current case, although laboratory tests for opportunistic infection like *P. jiroveci* were negative; more likely, there was no probability of respiratory symptoms due to pulmonary alveolar proteinosis (PAP). In this study, PAP was mentioned as differential diagnosis and pediatric pulmonologist did not advise BAL to demonstrate PAP. Based on the pathology results, PAP is usually categorized into three types including: (1) congenital, (2) autoimmune and (3) secondary. The pivotal role of CD40 ligand for activation of macrophages was established [14] that indicated macrophage dysfunction as the main cause of PAP [15]. On the other hand, mutation in *CD40L* gene can lead to malfunction in activation of macrophages that catabolize surfactant within alveoli results in PAP [16].

In the current study, although the main causative agent of respiratory symptoms was not characterized, concerning the lack of strong evidences of PAP and elevated level of CRP, it seems that the symptoms may be related to microbial agents that became opportunistic due to immunodeficiency resulting from functional mutation in *CD40L* gene.

In this case, mutation in *CD40L* gene (NM_000074: exon5: c.T464C) was determined by WES and described as the reason of XHIGM. However, although flow-cytometry is able to determine the functionality of CD40L, WES together with available sequencing approaches can provide deep screen of genes that are responsible for XHIGM. This mutation was introduced for the first time as a null mutation [17]. After that Prasad showed this mutation from a patient with XHIGM in Australia [18]. Nevertheless, the cases of XHIGM who carry this mutation are very rare. However, our case is the third XHIGM patient who had this functional mutation. Prasad and colleagues represented 11 cases with phenotypic features caused by XHIGM. In accordance with this study, in our case, mutation c.T464C in exon5 was the reason of XHIGM.

Nevertheless, as mentioned above, it seems that despite the precise follow up of these patients, most of HIGM patients do not survive until the second decades of their life [1]. Importantly, hematopoietic stem cell transplantation (HSCT) has recently introduced as a potential treatment of these patients. However, it is well-established that this kind of treatment has more suitable outcome, particularly after transplantation from a full matched donor [19].

This is the first study in Iran representing mutation in exon5 of the *CD40L* gene responsible for phenotypic features in XHIGM patient. In the other hand, XHIGM can lead to immunodeficiency that makes human body prone to infection.

Funding

There was no fund for the current study.

Disclosure of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to appreciate Dr. Hamed Mirjalali for editing the manuscript.

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