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ARAŞTIRMA RESEARCH ARTICLE

Long term follow-up of hyperimmunoglobulin M syndrome cases

Hiperimmünglobulin M sendromlu olguların uzun dönem izlemi*

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ABSTRACT

Objective: Hyperimmunoglobulin M (HIGM) syndromes are primary immunodeficiencies characterized by normal or elevated serum IgM levels with decreased levels of other immunoglobulin isotypes. Over the past decade rapid progress has been made in the molecular and genetic basis of HIGM and five distinct subgroups have been described.

Materials and Methods: Records of patients diagnosed as HIGM were retrospectively reviewed to describe the demographic characteristics, initial clinical presentation, age at onset and diagnosis and family history of immunodeficiency, in addition to basic immunological laboratory parameters, and information about follow-up and outcome of patients. Laboratory analyses included complete blood cell and differentials, serum immunoglobulin levels and immunophenotyping of peripheral blood lymphocytes. Flow cytometric analyses of CD40 and stimulated CD40 ligand (CD40L) expression were performed and compared to healthy controls.

ÖZET

Giriş: Hiperimmünglobulin M (HIGM) sendromu normal ya da yüksek serum IgM düzeylerinin varlığında diğer immünglobulin seviyelerinin düşüklüğü ile seyreden primer bir immünyetmezlik hastalığıdır. Son yıllarda bu hastalığın moleküler ve genetik temellerine ilişkin gelişmeler kaydedilmekte olup beş alt grup olarak tanımlanmıştır.

Gereç ve Yöntem: Kliniğimizde HIGM tanısı ile takip edilen hastalar demografik ve klinik özellikleri, tanı yaşı, aile öyküsü ve laboratuvar bulguları açısından retrospektif olarak değerlendirilmiştir. Olguların tam kan sayımları, periferik yayma incelemeleri, serum immünglobulin düzeyleri, lenfosit alt grup incelemeleri, CD40 ve sağlıklı kontrollere göre uyarılmış CD40 ligand (CD40L) gösterimleri çalışılmıştır.

Bulgular: Bu çalışmada 5 HIGM (4 erkek, 1 kız) olgusunun ortalama 7.2 yıl (median: 9, min-maks: 0.4-11.6 yıl) süreyle izlemi değerlendirilmektedir. Hastaların şikayetleri genellikle süt çocukluğu döneminde başlarken tanı yaşı 1.3-2.3 yıl (median: 2

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Results: We hereby describe 5 HIGM cases (1 female, 4 male) followed in our unit with a mean duration of 7.2 (median 9 years; min-max: 0.4-11.6) years. The age at diagnosis ranged between 1.3-2.3 years (median 2 years), whereas onset of symptoms was generally during infancy. Four cases were from consanguineous parents. Neither autoimmunity, lymphoma, neutropenia or ectodermal dysplasia were detected in any of cases. Evaluation of CD40 was available in four cases and showed normal expression. Stimulated CD40L expressions were found to be normal in the girl and remarkably lower than healthy controls in three boys. Lung imaging revealed bronchiectasis in three patients. All patients are under intravenous immunoglobulin treatment and antibiotic prophylaxis.

Conclusion: HIGM is associated with an increased risk of severe infections and chronic lung damage. Early-diagnosis and prompt initiation of intravenous immunoglobulin therapy is essential to prevent complications.

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Key words: CD40, CD40 ligand, hyperimmunoglobulin M syndrome, outcome, pediatrics

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Sonuç: HIGM sendromlu olgular sık infeksiyon geçirme ve kalıcı akciğer hasarı oluşması riskleriyle karşılaşmaktadır. Bunlardan korunmak için erken yaşta tanı konulması ve tedaviye bir an önce başlanması oldukça önem taşımaktadır.

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Anahtar kelimeler: CD40, CD40 ligand, hiperimmünglobulin M sendromu, klinik izlem, pediatri

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INTRODUCTION

Hyperimmunoglobulin M (HIGM) syndromes are primary immunodeficiencies characterized by normal or elevated serum IgM levels with decreased levels of other immunoglobulin isotypes. Rosen et al. described two brothers with recurrent infections in 1961[1]. Thereafter, Burtin reported similar patients who had low levels of IgG and elevated IgM[2]. These two reports were known to be the first clinical presentations of HIGM syndrome. Although this syndrome was originally termed as "dysgammaglobulinemia" in view of the dissociation between normal or elevated IgM and low-to-undetectable IgG and IgA, afterwards, a World Health Organization (WHO) working party named the syndrome immunodeficiency with HIGM in 1974[3].

The nature of the immune defects in HIGM remained elusive. For a long time, B-lymphocytes from HIGM patients were hypothesized to have an intrinsic inability to undergo immunoglobulin isotype switching[4]. Recently, as more genes were found to be mutated in the syndrome with different clinical presentations, it was suggested to change the name to "defective immunoglobulin class switch recombination". To date, five different genetic defects leading to HIGM have been identified[5]. Patients with these genetic defects leading to HIGM present with various clinical characteristics and outcomes. We hereby reported our experience based on clinical, laboratory features and outcome of HIGM cases followed in our unit for a mean duration of 7.2 years (median 9 years; min-max 0.4-11.6 years).

MATERIALS and METHODS

The diagnosis of HIGM was established in the presence of clinical symptoms including recurrent respiratory tract infections and dysgammaglobulinemia (normal or elevated levels of serum IgM in the presence of low levels

of serum IgA and IgG). All the records of patients suspected for a diagnosis of primary immunodeficiency were analyzed retrospectively who admitted to our unit in between years 2005-2009. The demographic characteristics, initial clinical presentation, age at onset of symptoms, age at diagnosis and presence of family history for immunodeficiency, in addition to basic immunological laboratory parameters were reviewed. The follow-up information and outcome of patients was also evaluated. Initial laboratory analyses included complete blood cell count with differentials. Serum immunoglobulin levels including IgG, IgA, IgM, IgE were determined by nephelometric method. The capacity to produce IgG type specific antibodies to commonly used childhood vaccines including hepatitis B virus vaccine and pneumococcal vaccine was determined by measuring antibody titers. Also isohemaglutinin titers in response to blood group antigens were determined in order evaluate IgM type antibody response. Immunophenotyping of peripheral blood lymphocytes (for T cells: CD3+, CD3+CD4+, CD3+CD8+; for B cells: CD19+, CD20+; for natural killer cells: CD16+CD56+) as well as CD40 expression on B lymphocytes with whole blood lysis method were evaluated by flow cytometry. CD40 ligand expression on T lymphocytes was analyzed in isolated peripheral blood mononuclear cells incubated for five hours with or without phorbol myristate acetate (PMA, 20 ng/mL final, Sigma) and Calcium ionophor (hemi-calcium salt, 300 ng/mL final, Sigma) as stimulant at 37°C and 5% CO_2[6].

RESULTS

Demographic Data

One female (P1), 4 male (P2-P5) patients were diagnosed as HIGM. The mean duration of follow up of these cases in our unit was 7.2 years. The median age of diagnosis was two years, which ranged between 1-23 years. The onset of symptoms was generally during infancy (6-24 months). The mean current age of the patients was 13.6 years ranging between 1.7-26.9 years. Parents of four cases have been reported to have consanguinity (P1-P4).

Clinical Evaluations

The most common presenting clinical feature detected in our HIGM cases was recurrent pneumonia. Out of five patients, three of them had a history of recurrent pneumonia, ≥ 2 times in their lives (P1, P3 and P5). Recurrent otitis was observed in two of patients (P1, P3) with complicating perforation of the tympanic membranes. Recurrent lymphadenitis was another clinical presentation with accompanying fever, tenderness, erythema and indurations localized to cervical region (P2). P4 was the only case in which no symptoms developed and diagnosis was established due a positive family history of a sibling with HIGM (P3).

On physical examination, the noteworthy finding was failure to thrive in all cases except in asymptomatic P4. Auscultation of the chest revealed bilateral crackles in four cases (P1-P3, P5). We observed no associated ectodermal dysplasia in our cases suggesting NF-κB essential modulator (NEMO) defect. P1 was formerly operated for the removal of left lower lobe of the lung and then re-lobectomized for the left lower lobe in the following years before admission to our unit when she was 15 years of age. P5 had died at the age of 1.7 years due to respiratory failure.

Laboratory Evaluations

Complete blood counts with differentials, as well as absolute neutrophil and lymphocyte counts were within normal ranges. Only P3 demonstrated low hemoglobin levels. Platelet counts were also within normal limits.

Serum IgM levels of all patients were above +2 standard deviation (SD) for age ranging between 845-2240 g/L. On the other hand patients had low levels of other immunoglobulin isotypes based on -2 SD for age. The values for IgG, IgA and IgE were presented in Table 1. High IgM levels persisted during the follow-up period while patients were receiving their regular intravenous immunoglobulin (IVIG) replacement.

Table 1. Demographic and laboratory data of cases with HIGM syndrome

	P1	P2	P3	P4	P5*
Demographics					
Gender	Female	Male	Male	Male	Male
Current age (years)	26.9	12.2	16.1	11.0	1.7
Age of first complaints (months)	6	6	24		6
Age at diagnosis (years)	23	1	4.5	2	1.3
Presenting findings	Recurrent otitis and pneumonia	Recurrent lymphadenitis	Recurrent upper respiratory tract infections and pneumonia	Family history	Recurrent pneumonia
Follow-up duration (years)	3.9	11.2	11.6	9.0	0.4
Consanguinity	+	+	+	+	-
Immunoglobulins					
At diagnosis					
IgM (g/L)	1270	1120	2240	1040	845
IgG (g/L)	< 142	9	6	147	27
IgA (g/L)	< 25	< 25	< 25	< 25	< 25
IgE (IU/mL)	< 1	< 1	< 1	< 1	< 1
Currently					
IgM (g/L)	883	716	942	2140	1760
IgG (g/L)	1280	614	1710	1620	32
IgA (g/L)	< 25	< 25	< 25	< 25	< 25
IgE (IU/mL)	< 1	< 1		< 1	
Laboratory					
Anti-HBsAg titers (> 10 mIU/mL)	Positive	n/a	n/a	Positive	Negative
Isohemagglutinin titers (> 1/8)	Positive	Positive	n/a	n/a	n/a
Pnomococcal response (4x increase)	Positive	n/a	n/a	n/a	n/a
Tuberculin skin test reactivity (mm)	8	4	1	1	n/a
Imaging of lungs	Bronchiectasis, lobectomy	Mosaic perfusion & fibrotic tractions	Bronchiectasis	Ground glass appearance	Destructive chronic lung disease

* P5 was deceased at the age of 1.7 years-old.
n/a: Not available.

Subset analyses of lymphocytes revealed normal numbers of CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD20+ and CD16+CD56+ cells. Evaluation of CD40 was available in P1-P4 and showed normal expression. Stimulated CD40L expressions were found to be normal in P1 and remarkably lower than healthy controls in three patients (P2-P4).

The available anti-hepatitis B, anti-pneumococcal IgG and isohemagglutinin IgM titers were presented in Table 1, as well as intradermal tuberculin skin testing results. Autoimmunity, lymphoma and deterioration of liver enzymes were not detected in any of our cases. Bronchiectasis and chronic changes in the lung parenchyma were the most remarkable findings of lung imagings observed in all cases to some extent.

During follow-up all patients were receiving IVIG replacement at a dose of 0.5-0.8 g/kg/dose every three-four weeks and trimethoprim-sulfamethoxazole at a dose of 1 x 4 mg/kg/day prophylactically.

DISCUSSION

Patients with HIGM have markedly reduced levels of serum IgG, IgA, IgE and normal to elevated serum IgM levels in the presence of normal counts of circulating B cells. It has been strongly suggested that a defect in immunoglobulin class switch recombination and/or somatic hypermutation is responsible in the pathogenesis of this syndrome. Genetic causes underlying the HIGM are variable and can be predicted by the clinical, laboratory and immunophenotypical features of the patients in addition to confirmation of molecular defects by the sequence analyses using cDNA or genomic DNA. The described five distinct molecular defects and their representing clinical features of HIGM are summarized in Table 2.

The X-linked form of HIGM (XHIGM) accounts for 65-70% of the HIGM phenotype. Onset of symptoms mostly appears during infancy with upper and lower respiratory tract infections in affected males. Patients with XHIGM are susceptible to interstitial pneumonia caused by *Pneumocystis carinii* pneumonia (PCP) and to diarrhea caused by cryptosporidium that may contribute to sclerosing cholangitis[7,8]. Lymph nodes of patients with CD40L deficiency lack germinal centers resulting in lymphoid hypoplasia[9]. Autoimmune manifestations are quite common and include thrombocytopenia, seronegative arthritis, and inflammatory bowel disease[7]. Neutropenia is present in two out of three patients and does not seem to be associated with autoantibodies[7]. The clinical diagnosis has to be confirmed by demonstrating a defect in the expression of CD40L by activated peripheral blood $CD4^+$ lymphocytes by flow cytometry[10]. A multicenter European study reported that only 20% of the patients survived beyond 25 years of age and the general causes of death included infections during early life, liver disease, and malignancies[11]. In our cohort, P3-P5 are assumed to have CD40L deficiency by their clinical and immunophenotypical features.

A limited number of cases have been reported as autosomal recessive form of HIGM due to CD40 deficiency[12,13]. The CD40-deficient patients identified to date were found to have a complete lack of CD40 expression on the surface of B lymphocytes and monocytes. None of our patients had a deficiency of CD40 expression during analyses. Another form of autosomal recessive HIGM due to Activation-Induced Cytidine Deaminase (AID) deficiency has been described in patients with normal CD40L with increased susceptibility to bacterial, but not opportunistic infections[14,15]. The diagnosis of AID deficiency should be considered in patients with abnormal serum Ig levels, suggesting the HIGM phenotype in the presence of normal

Table 2. Laboratory and clinical features of five molecular defects leading to HIGM

Molecular defect	CD40L	CD40	AID	UNG	NEMO
Prevalence (%)	70	10	< 1	5	1
Inheritance	X-linked	Autosomal recessive	Autosomal recessive	Autosomal recessive	X-linked
Onset of symptoms (years)	0.5-1	2-10	0.5-1	0.3-20	0.5-1
Opportunistic infections	+	-	+	-	-
Lymphoid hyperplasia	-	+	-	+	-

HIGM: Hyperimmunoglobulin M, CD40L: Cluster of differentiation 40 ligand, CD40: Cluster of differentiation 40, AID: Activation-induced cytidine deaminase, UNG: Uracil DNA glycosylase, NEMO: Nuclear factor-κB essential modulator.

CD40L expression and should be confirmed by the molecular detection of mutations within the AID gene. Our female case (P1) seems to be a candidate for AID deficiency due to normal CD40 and stimulated CD40L expressions.

On the other hand, the clinical phenotype of uracil DNA glycosylase (UNG) deficiency resembled patients with AID deficiency, including susceptibility to bacterial infections, lymphoid hyperplasia, increased serum IgM concentrations, and profoundly decreased serum IgG and IgA levels with normal numbers of B and T cell subsets. UNG gene mutations were described for the first time in HIGM patients with normal CD40L, CD40, AID analyses[16]. Due to presence of persistent lymphoid hyperplasia in P2, UNG deficiency has to be kept in mind as the underlying molecular defect of this patient.

A subgroup of hypohidrotic (anhidrotic) ectodermal dysplasia (EDA) patients is characterized by dysgammaglobulinemia with decreased serum IgG and decreased or elevated IgM and IgA levels. The gene responsible for this X-linked form of HIGM has been identified as the nuclear factor-κB essential modulator (NEMO)[17]. None of our cases presented with ectodermal dysplasia.

Our patients demonstrated variable clinical presentations of HIGM in both genders despite the small number of cases. This may be related to the higher rate of consanguineous marriages in Turkey, which is estimated to be approximately 25%[18,19]. Regarding this fact, pattern of autosomal recessive inheritance is expected to be higher in our country.

It is important to overcome the infections and provide a better prognosis for these patients. IVIG replacement at doses of 400-600 mg/kg per month in all sub-types of HIGM should be established as soon as the diagnosis is confirmed. These cases should also receive prophylaxis with trimethoprim-sulfamethoxazole. In addition, patients with persistent severe neutropenia are candidates for treatment with granulocyte colony-stimulating factor (G-CSF) transfusions[20]. Hematopoietic stem cell transplantation (HSCT) is suggested to be a therapeutic option for the CD40 deficient X-linked HIGM patients. Gennery et al. reported that 58% of 38 X-linked HIGM patients had been cured who was performed hematopoietic stem cell transplantation in eight European countries. In that series, emerging lung disease was the most significant adverse risk factor[21]. Long term adverse effects and outcome of hematopoietic stem cell transplantation in HIGM cases should also be further studied.

Regarding the high parental consanguinity in our country, family members should also be examined and evaluated for HIGM. In our experience, P4 was diagnosed due to sibling history, which enabled us to initiate IVIG therapy prior to development of infections. Therefore, this patient had normal physical examination findings, lung functions and normal lung imaging at onset.

In conclusion, prompt evaluation of cases with recurrent upper and lower respiratory tract infections for the diagnosis of HIGM is essential, and appropriate treatment is crucial to provide a better outcome for these patients. In a country possessing a high parental consanguinity, rarely seen forms of immunodeficiency syndromes should be kept in mind by pediatricians.

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