Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

Journal homepage: http://www.e-jmii.com

Original Article

Evaluation of Immunoglobulin Levels and Infection Rate in Patients with Common Variable Immunodeficiency After Immunoglobulin Replacement Therapy

Mahin Salehzadeh[a], Asghar Aghamohammadi[a,b], Nima Rezaei[a,b]*

[a]Department of Pediatrics, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran.
[b]Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran.

BACKGROUND/PURPOSE: Common variable immunodeficiency (CVID) is characterized by decreased serum levels of IgG and increased susceptibility to recurrent infections. The aim of this study was to evaluate the IgG subclass levels of CVID patients.
METHODS: Twenty-four CVID patients who had been under regular intravenous immunoglobulin replacement therapy for 96.13 ± 54.83 months were enrolled in this study. Serum IgG and IgG subclass levels, and clinical outcomes for these patients were evaluated after this period of treatment.
RESULTS: Mean serum IgG levels were significantly increased from 272.91 ± 185.58 mg/dL at the time of diagnosis to 455.29 ± 200.23 mg/dL after treatment, while there was no significant difference in the serum levels of IgM and IgA. Decreased serum levels of IgG1 were detected in 75% of the patients studied. Decreased serum levels of IgG2, IgG3 and IgG4 were also detected in 6, 11, and 11 patients, respectively. All patients experienced recurrent infectious diseases either before, or after, diagnosis.
CONCLUSION: Although serum IgG levels in the patients significantly increased after regular intravenous immunoglobulin replacement therapy, serum IgM and IgA levels remained diminished over time. Furthermore, a number of cases had low levels of IgG subclasses, in spite of normal total IgG levels, which could explain why some patients had continued infections, even after immunoglobulin replacement therapy.

KEYWORDS: common variable immunodeficiency, immunoglobulin, IgG subclasses, infection

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of primary immunodeficiency diseases characterized by hypogammaglobulinemia and an increased susceptibility to recurrent infections, autoimmunity, and malignancy.[1–6] Poor antibody responses to protein or polysaccharide antigens are a prominent finding in a particular group of patients.[7–9] Several immunological mechanisms that affect B cells, T cells, and dendritic cells have been described in CVID,[10–13] but the basic molecular defect that causes the disease is still unknown.[14,15]

*Corresponding author. Children's Medical Center, 62 Qarib Street, Keshavarz Boulevard, Tehran 14194, Iran.

E-mail: nima_rezaei@farabi.tums.ac.ir or rezaei_nima@hbi.ir

Article History:
Received: Feb 5, 2009
Revised: Feb 25, 2009
Accepted: Mar 25, 2009

Copyright © 2010 Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. All rights reserved.

Immunoglobulin replacement therapy is the treatment of choice for patients with hypogammaglobulinemia as it decreases the incidence and severity of infections, and improves the quality of life of these patients.[16–23] However, in spite of regular immunoglobulin infusions, the serum IgG levels of some patients may not reach normal levels.

This study was performed to evaluate both serum IgG and IgG subclass levels in a group of CVID patients who were undergoing intravenous immunoglobulin (IVIG) replacement therapy for at least 2.5 years. Clinical outcomes were also evaluated.

Patients and Methods

Patients

This study was approved by the Ethical Committee of Tehran University of Medical Sciences. Written informed consent was obtained from all patients before sampling.

Twenty-four patients with CVID, who had been diagnosed and treated in the Division of Allergy and Clinical Immunology of Children's Medical Center Hospital, were enrolled in this study. The diagnosis of CVID was based on standard criteria introduced by the European Society for Immunodeficiencies and the Pan-American Group for Immunodeficiency, and was also compatible with the new classification of primary immunodeficiency diseases introduced by the International Union of Immunological Societies.[15,24] These include reductions in at least two of three serum immunoglobulin levels (IgG, IgM, IgA) by two standard deviations from the normal mean values for age, and genetic exclusion of other well-defined antibody deficiencies. In patients with low numbers of B cells, mutation analysis of candidate genes for X-linked (*BTK*) and autosomal recessive (*IGHM, CD79A, CD79B, IGLL1, BLNK*) agammaglobulinemia was performed.[25] The *SH2D1A* gene was analyzed in some susceptible male cases of X-linked lymphoproliferative syndrome. In some patients with normal or elevated serum IgM levels, the genes for *CD40L*, *CD40*, *AID* and *UNG* were sequenced due to suspicion of immunoglobulin class-switch-recombination deficiencies.[6] Patients under two years of age were excluded from this study because of a possible diagnosis of transient hypogammaglobulinemia. All patients were under regular IVIG replacement therapy (300–600 mg/kg every 3–4 weeks) and followed-up for a period of at least 30 months.

Immunophenotyping

Immunophenotyping of fresh blood was performed by flow cytometry. The percentage of cells expressing CD markers was used as the unit of measurement. CD3+ T cells and CD19+ B cells were assessed. The percentages of CD3+CD4+ T cells and CD3+CD8+ T cells were also measured (Serotec, Oxford, United Kingdom).

Immunoglobulin assay

The first immunoglobulin assay was performed at the time of diagnosis. Subsequent assays were performed 3–4 weeks after IVIG replacement therapy, and just prior to the next scheduled immunoglobulin infusion. Serum immunoglobulin levels (IgG, IgM, and IgA) and IgG subclasses (IgG1, IgG2, IgG3, and IgG4) were measured by nephelometry (The Binding Site, Birmingham, UK).

Period of disease

The course of disease was divided into pre- and post-diagnosis, when IVIG replacement therapy was administered to the patients. The time before diagnosis was the period between age of onset and age at diagnosis (diagnosis delay). The time after diagnosis was the period between age at diagnosis and the age at the time of the study (follow-up period). The number of infections, and hospital admissions due to serious infections were calculated (per patient per year) and compared between the two periods. Recurrent infections were defined as more than three episodes of infection in the same organ.

Statistical analysis

Data analysis was performed using the SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Data presented as the mean ± standard deviation and as median values (minimum–maximum). The χ^2 test and Student's t test were performed for some comparisons between the two periods. A p value of < 0.05 was considered significant.

Results

Patient characteristics

Twenty-four patients with CVID (17 male and 7 female) were investigated in this study. The median age at the time of diagnosis was 102.5 months (2–43 years), with a median diagnosis delay of 63.5 months (3–477 months). The

patients were followed-up for 96.13±54.83 months (median, 78 months; range, 32–225 months), giving a total of 2,307 patient-months (192.25 years). The mean age of patients at the time of the study was 19.49±12.60 years (median, 16.17 years; range, 5–49 years).

Immunophenotyping

Lymphocyte subpopulation studies revealed that the number of CD3+ T cells was within the normal range according to age (Figure 1). However, five patients had decreased percentages of CD19+ B cells (between 2% and 6%). A CD3+CD4+ T cells to CD3+CD8+ T cells of >1 was found in 13 of the cases, while a reverse ratio was found in the remaining 11 cases (45.8%).

Serum immunoglobulin levels

The mean serum IgG concentration of the patients was 272.91±185.58 mg/dL at the time of diagnosis, while median serum IgM and IgA levels were 28.5 mg/dL (range, 0–140 mg/dL) and 5.5 mg/dL (range, 0–98 mg/dL), respectively.

Several immunoglobulin assays were performed after IVIG replacement therapy and the mean serum immunoglobulin levels calculated. Mean serum IgG was significantly increased to 455.29±200.23 mg/dL ($p<0.001$), while there was no significant difference in IgM ($p=0.653$) or IgA ($p=0.196$) concentrations. The median serum IgM and IgA levels of the patients after IVIG replacement therapy were 23.5 mg/dL (range, 0–150 mg/dL) and 5 mg/dL (range, 0–60 mg/dL), respectively (Figure 2).

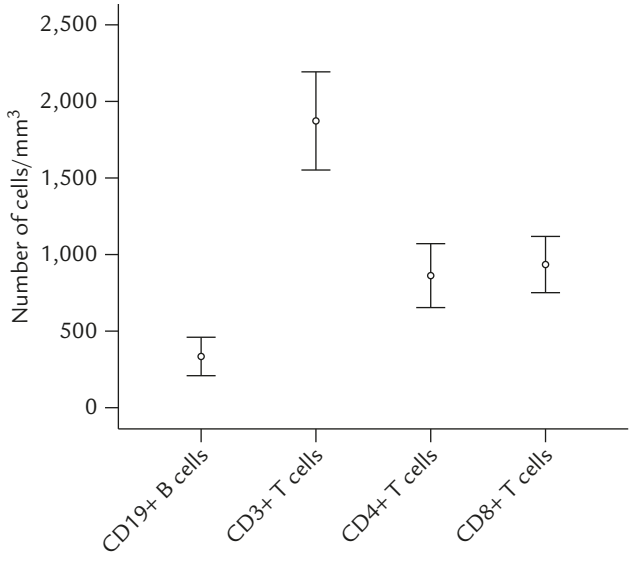

Figure 1. Absolute number of B and T cell subpopulation in patients.

IgG subclass levels

A cross-sectional evaluation of IgG and its subclasses in all cases revealed that half of the cases had a normal range of IgG concentration according to age. The mean serum IgG level was 613.25±299.72 mg/dL, which was significantly higher than the level at the time of diagnosis ($p<0.0001$). Although 11 patients had IgG serum levels of more than 600 mg/dL, decreased serum levels of IgG1 were detected in 18 cases (75%), including five cases with a normal range of total IgG. Mean serum IgG1 levels were 348.46±206.02 mg/dL. Decreased serum levels of IgG2 were found in six patients, while 11 patients had reduced serum levels of IgG3 for their age. Decreased serum levels of IgG4 were also found in 11 cases (Table 1). The mean serum IgG2, IgG3, and IgG4 levels were 215.51±104.63 mg/dL, 37.79±31.59 mg/dL, and 1.11±0.71 mg/dL, respectively.

Clinical outcome

Infection was the most common clinical manifestation, either before diagnosis, or after IVIG replacement therapy.

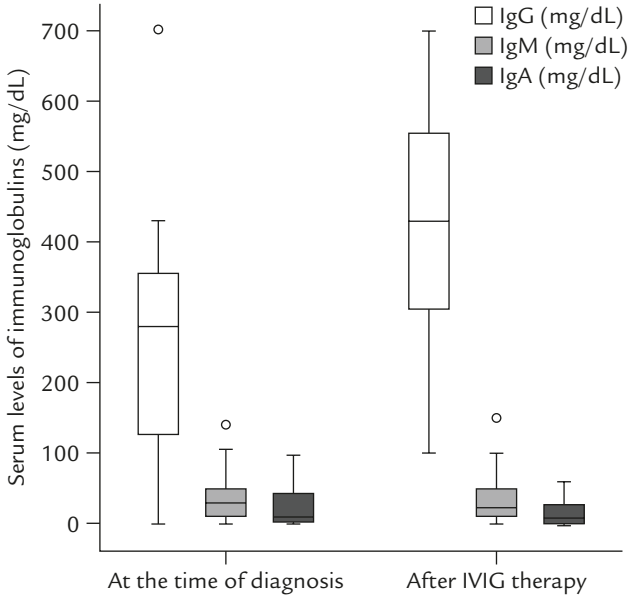

Figure 2. Box plot figure of serum immunoglobulin levels at the time of diagnosis and 3–4 weeks after the intravenous immunoglobin replacement therapy. IVIG=intravenous immunoglobin replacement therapy.

Table 1. Serum IgG subclass levels of common variable immunodeficiency patients ($n=24$)

Patient no.	Sex	Age (yr)	IgG (mg/dL)	IgG1 (mg/dL)	IgG2 (mg/dL)	IgG3 (mg/dL)	IgG4 (mg/dL)
1	M	47	639.10	243.80[a]	352.10	27.55[a]	1.09
2	F	16	837.00	365.50[a]	407.80	57.83	1.58
3	F	7	836.20	315.60[a]	366.50	19.80	1.03
4	M	49	1,099.00	693.30	222.70	1.12[a]	1.59
5	M	9	1,376.00	956.50	227.10	103.90	1.73
6	M	49	426.70[a]	206.90[a]	73.36[a]	26.17[a]	0.70[a]
7	M	14	434.50[a]	177.10[a]	77.94[a]	22.62[a]	3.11
8	M	17	990.80	685.30	197.80	87.07	0.61[a]
9	M	20	354.40[a]	217.70[a]	83.06[a]	30.19	1.58
10	M	26	433.40[a]	218.00[a]	230.70	17.66[a]	0.43[a]
11	F	31	352.10[a]	266.80[a]	108.30[a]	24.11[a]	0.53[a]
12	F	18	536.30[a]	234.40[a]	303.10	35.95	0.41[a]
13	M	9	578.80[a]	320.90[a]	233.60	14.85[a]	2.19
14	M	16	560.70[a]	315.80[a]	243.90	18.33[a]	0.97[a]
15	M	9	456.80[a]	211.50[a]	141.80	77.62	1.21
16	F	18	760.90	383.40[a]	276.90	24.07[a]	1.12[a]
17	M	21	130.00[a]	150.00[a]	95.00[a]	0.00[a]	0.00[a]
18	M	5	392.00	209.70[a]	156.00	11.78	0.53[a]
19	M	16	22.20[a]	16.40[a]	11.57[a]	0.00[a]	0.00[a]
20	M	10	747.10	418.00	325.10	13.11	1.51
21	F	12	692.30	438.60	284.50	64.37	1.10
22	F	22	545.90[a]	339.60[a]	249.90	94.72	1.05
23	M	12	837.40	576.60	307.60	51.28	0.88[a]
24	M	16	678.30	401.60[a]	196.00	82.77	1.81

[a]Decreased serum levels according to age.

The most commonly encountered infections were pneumonia (21 patients), diarrhea (21 patients), sinusitis (19 patients), and otitis media (16 patients). Other, less frequent, infections were conjunctivitis (8 patients), cutaneous infections (8 patients), and five patients each with superficial abscesses, mucocutaneous candidiasis, and septic arthritis. Bronchiectasis was documented in seven patients. While recurrent infections were significantly decreased in comparison with the period before diagnosis after IVIG replacement therapy, the number of patients who had upper respiratory tract infections did not significantly differ between the two time periods (Table 2). The incidence of hospital admission due to severe infection significantly reduced from 1.21/patient/year pre-diagnosis, to 0.125/patient/year during IVIG replacement therapy ($p=0.008$).

Discussion

CVID is a heterogeneous group of disorder with different immunological phenotypes.[1–3,26,27] Recurrent infectious complications before diagnosis are the most common manifestations, and some patients continue to experience such infections after treatment. In this survey, we studied both the serum IgG and IgG subclass levels in CVID patients undergoing regular IVIG replacement therapy for more than 30 months.

Hypogammaglobulinemia is the main feature of CVID. Thus immunoglobulin replacement therapy, either intravenous or subcutaneous, is recommended as the approved treatment in this group of patients.[6] As shown in this study, such treatment decreases the rate of recurrent and severe infections and consequent hospitalization.[16–23]

Table 2. Comparison of respiratory and gastrointestinal infections in common variable immunodeficiency patients before and after diagnosis

Infections	Before diagnosis	After diagnosis	*p*
Otitis media	14 (58.3)	9 (37.50)	0.240
Recurrent otitis media[a]	11 (45.8)	1 (4.17)	0.002
Sinusitis	17 (70.8)	11 (45.80)	0.140
Recurrent sinusitis[a]	6 (25.0)	1 (4.17)	0.048
Pneumonia	21 (87.5)	6 (25.00)	<0.001
Recurrent pneumonia[a]	10 (41.7)	1 (4.17)	0.006
Diarrhea	20 (83.3)	7 (29.20)	0.004
Recurrent diarrhea[a]	12 (50.0)	1 (4.17)	0.001

[a]More than three episodes of infection.

Although immunoglobulin replacement therapy has changed the spectrum of diseases, several medical complications are still observed in these patients. In this study, while recurrent infections were significantly decreased after IVIG replacement therapy, there was no significant difference in the number of patients who had upper respiratory tract infections between the two time periods. Pneumonia was the most common manifestation, followed by diarrhea, sinusitis, and otitis media. Although CVID patients are susceptible to recurrent infections at different sites, it seems that the respiratory and gastrointestinal systems are more involved.[1,2,28]

Twenty one out of 24 CVID patients had recurrent pneumonia, and one third of these cases developed bronchiectasis. Although the rate of pneumonia decreased significantly after IVIG replacement therapy, respiratory infections should be considered as a common medical problem in CVID patients, and failure to provide adequate immunoglobulin replacement therapy could lead to early bronchiectasis.

In this study, the patients only rarely experienced recurrent upper respiratory tract infections after IVIG therapy, while there was no significant difference in number of patients who had a single episode of either otitis media or sinusitis between the two periods. It seems that upper respiratory tract infections continue to be important problems in hypogammaglobulinemic patients, even after treatment with immunoglobulin substitution. It has been shown that such treatment cannot eradicate such infections after late diagnosis, possibly due to structural damage in the mucociliary system.[29,30]

Nearly all the CVID patients in this study had decreased serum immunoglobulin levels before treatment. Although serum IgG levels in these patients significantly increased after regular IVIG replacement therapy, the levels of IgM and IgA were stable over time. However, it seems that some patients do not reach normal levels, and have a deficit of IgG and/or IgG subclasses. A number of cases had decreased IgG subclass levels, in spite of normal total IgG levels, which shows the importance of IgG monitoring after IVIG administration. Such deficiencies in immunoglobulin levels could explain why a number of patients continue to have infections, even after immunoglobulin replacement therapy.

It is important to consider CVID in any patient, even pediatric cases, with a history of recurrent infections. Assessment of the immune system, including measurement of serum immunoglobulin levels, IgG subclass levels, antibody responses to polysaccharide antigens, and lymphocyte subset enumeration should be performed for such patients. The serum immunoglobulin levels of the patients should also be checked regularly; this helps physicians to manage these patients.

Acknowledgments

This work was supported by Tehran University of Medical Sciences and Health Services grant. The authors are very grateful to our colleagues in the Children's Medical Center for their kind help and advice in the laboratory, and to all the patients and their families for their kind collaboration in this study.

References

1. Aghamohammadi A, Farhoudi A, Moin M, Rezaei N, Kouhi A, Pourpak Z, et al. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. *Clin Diagn Lab Immunol* 2005;12:825–32.
2. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin Immunol* 1999;92:34–48.
3. Hammarstrom L, Vorechovsky I, Webster D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol* 2000;120:225–31.
4. Rezaei N, Aghamohammadi A, Moin M, Pourpak Z, Movahedi M, Gharagozlou M, et al. Frequency and clinical manifestations of patients with primary immunodeficiency disorders in Iran: update from the Iranian primary immunodeficiency registry. *J Clin Immunol* 2006;26:519–32.
5. Spickett GP. Current perspectives on common variable immunodeficiency (CVID). *Clin Exp Allergy* 2001;31:536–42.
6. Aghamohammadi A, Lougaris V, Plebani A, Miyawaki T, Durandy A, Hammarström L. Predominantly antibody deficiencies. In: Rezaei N, Aghamohammadi A, Notarangelo LD, eds. *Primary immunodeficiency diseases: definition, diagnosis and management*. Berlin Heidelberg: Springer-Verlag, 2008:97–130.
7. Glocker E, Ehl S, Grimbacher B. Common variable immunodeficiency in children. *Curr Opin Pediatr* 2007;19:685–92.
8. Rezaei N, Aghamohammadi A, Siadat SD, Nejati M, Ahmadi H, Moin M, et al. Serum bactericidal antibody response to serogroup C polysaccharide meningococcal vaccination in children with primary antibody deficiencies. *Vaccine* 2007;25: 5308–14.
9. Rezaei N, Aghamohammadi A, Siadat SD, Moin M, Pourpak Z, Nejati M, et al. Serum bactericidal antibody responses to meningococcal polysaccharide vaccination as a basis for clinical classification of common variable immunodeficiency. *Clin Vaccine Immunol* 2008;15:607–11.
10. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+) IgM(–)IgD(–)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. *Blood* 2002;99:1544–51.
11. Vodjgani M, Aghamohammadi A, Samadi M, Moin M, Hadjati J, Mirahmadian M, et al. Analysis of class-switched memory B cells in patients with common variable immunodeficiency and its clinical implications. *J Invest Allerg Clin Immunol* 2007;17: 321–8.
12. Nourizadeh M, Aghamohammadi A, Moazzeni SM, Mahdavi M, Rezaei N, Hadjati J. High production of IL-18 by dendritic cells induced by sera from patients with primary antibody deficiency. *Iran J Allergy Asthma Immunol* 2007;6:59–65.
13. Ravanbakhsh M, Sarafnejad A, Aghamohammadi A, Kardar GA, Asgarian Omran H, Atarod L, et al. CD40 ligand expression on stimulated T-helper lymphocytes in patients with common variable immunodeficiency. *Iran J Allergy Asthma Immunol* 2007; 6:129–35.
14. Webster ADB. Clinical and immunological spectrum of common variable immunodeficiency (CVID). *Iran J Allergy Asthma Immunol* 2004;3:103–13.
15. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, et al. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *J Allergy Clin Immunol* 2007;120:776–94.
16. Hedderich U, Kratzsch G, Stephen W, Dichtelmuller H, Olischlager K, Heimpel H. Immunoglobulin substitution therapy in a patient with primary hypogammaglobulinaemia and anti-IgA antibodies. *Clin Allergy* 1986;16:339–44.
17. Busse PJ, Razvi S, Cunningham-Rundles C. Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. *J Allergy Clin Immunol* 2002;109:1001–4.
18. Quartier P, Debre M, De Blic J, de Sauverzac R, Sayegh N, Jabado N, et al. Early and prolonged intravenous immunoglobulin replacement therapy in childhood agammaglobulinemia: a retrospective survey of 31 patients. *J Pediatr* 1999;134: 589–96.
19. Aghamohammadi A, Moin M, Farhoudi A, Rezaei N, Pourpak Z, Movahedi M, et al. Efficacy of intravenous immunoglobulin on the prevention of pneumonia in patients with agammaglobulinemia. *FEMS Immunol Med Microbiol* 2004;40: 113–8.
20. Pourpak Z, Aghamohammadi A, Sedighipour L, Farhoudi A, Movahedi M, Gharagozlou M, et al. Effect of regular intravenous immunoglobulin therapy on prevention of pneumonia in patients with common variable immunodeficiency. *J Microbiol Immuno Infect* 2006;39:114–20.
21. Bayrakci B, Ersoy F, Sanal O, Kilic S, Metin A, Tezcan I. The efficacy of immunoglobulin replacement therapy in the long-term follow-up of the B-cell deficiencies (XLA, HIM, CVID). *Turk J Pediatr* 2005;47:239–46.
22. Gardulf A, Bjorvell H, Gustafson R, Hammarstrom L, Smith CI. The life situations of patients with primary antibody deficiency untreated or treated with subcutaneous gammaglobulin infusions. *Clin Exp Immunol* 1993;92:200–4.
23. Atarod L, Aghamohammadi A, Moin M, Kanegane H, Rezaei N, Rezaei Kalantari K, et al. Successful Management of Neutropenia in a Patient with CD40 Ligand Deficiency by Immunoglobulin Replacement Therapy. *Iran J Allergy Asthma Immunol* 2007; 6:37–40.
24. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). *Clin Immunol* 1999;93: 190–7.

25. Aghamohammadi A, Fiorini M, Moin M, Parvaneh N, Teimourian S, Yeganeh M, et al. Clinical, immunological and molecular characteristics of 37 Iranian patients with X-linked agammaglobulinemia. *Int Arch Allergy Immunol* 2006;141: 408–14.
26. Cunningham-Rundles C. Common variable immunodeficiency. *Curr Allergy Asthma Rep* 2001;1:421–9.
27. Di Renzo M, Pasqui AL, Auteri A. Common variable immunodeficiency: a review. *Clin Exp Med* 2004;3:211–7.
28. Khodadad A, Aghamohammadi A, Parvaneh N, Rezaei N, Mahjoob F, Bashashati M, et al. Gastrointestinal manifestations in patients with common variable immunodeficiency. *Dig Dis Sci* 2007;52:2977–83.
29. Favre O, Leimgruber A, Nicole A, Spertini F. Intravenous immunoglobulin replacement prevents severe and lower respiratory tract infections, but not upper respiratory tract and non-respiratory infections in common variable immune deficiency. *Allergy* 2005;60:385–90.
30. Aghamohammadi A, Moazzami K, Rezaei N, Karimi A, Movahedi M, Gharagozlou M, et al. ENT manifestations in Iranian patients with primary antibody deficiencies. *J Laryngol Otol* 2008;122: 409–13.