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Clinical and Laboratory Evaluation of Periodically Monitored Turkish Children with IgG Subclass Deficiencies

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SUMMARY IgG subclass deficiencies are common immune system disorders during childhood. The aim of this retrospective study was to review clinical findings and laboratory results of patients with IgG subclass deficiencies in order to determine the changes in serum IgG subclass levels during follow-up, the percentage and time span until normalization of the IgG subclass levels to age-corresponding normal levels, the type of infections incurred and the benefits of prophylaxis. Among the 59 pediatric patients reviewed, the most frequent defect was an IgG3 subclass deficiency (77%). Nine percent of the patients had an isolated IgG2 deficiency and 14% had an IgG2+G3 deficiency. The most common clinical presentations were recurrent upper respiratory tract infections, followed by pneumonia, acute gastroenteritis and urinary tract infections. Atopy was present in 15% of the patients. Ninety percent of the patients were given a prophylactic treatment (benzathine penicillin, oral antibiotics, oral bacterial lysate or intravenous immunoglobulin). The frequency of recurrent infections decreased from 13.4 ± 7.4 per year to 5.7 ± 3.9 in patients receiving a prophylactic regimen. Serum IgG subclass levels reached normal ranges in 30% of the patients in the IgG3 deficiency group and in 35.7% of the patients in the IgG2+G3 deficiency group. Patients with an isolated IgG2 deficiency did not reach age-related normal levels during the study period. Our study shows that IgG subclass levels may normalize in 30 to 40% of patients at about 6 years of age. We emphasize the need of monitoring IgG levels together with the clinical symptomatology in affected individuals and initiate preventive measures when appropriate.

Recurrent infections in children are a substantial cause of morbidity and hospitalization worldwide. Several studies have demonstrated that a remarkably high proportion of children with recurrent infections have abnormalities in the humoral immune system. Since Schur *et al.*[1] described immunoglobulin G (IgG) subclass deficiencies in three patients with pyogenic pulmonary infections in 1970, several reports have shown the association between recurrent infections and low levels of IgG subclasses and/or a lack of a specific antibody response.

An isolated/selective IgG subclass deficiency can be defined as a deficiency in one or more IgG subclasses (more than two standard deviations below the mean of healthy individuals of the same age) with normal or near normal IgG concentrations.[2] IgG can be subdivided biochemically into four distinct subclasses. The relative proportion of each of the IgG subclasses is generally constant within the total amount of IgG present: IgG1 constitutes 66%; IgG2, 24%; IgG3, 7% and IgG4, 3% of the total IgG.[3] During normal immunological maturation the synthesis of IgG1 and IgG3 occurs before the synthesis of IgG2 and IgG4. The age at which each of the IgG subclasses reaches adult levels varies and

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each age group in childhood has its own normal levels.[4-6] Since IgG1 is the predominant serum IgG subclass, deficiency of IgG1 can not generally occur without a decrease in total serum IgG, in which instance the defect should be considered as a "common variable immunodeficiency".[7] Low levels of IgG3 are the most common IgG subclass abnormality reported in adults, whereas IgG2 deficiency is the most prevalent IgG subclass deficiency in pediatric patients. It is believed that IgG2 is responsible for the immune response to polysaccharide antigens, especially in the context of infections with *Streptococcus pneumoniae* and *Haemophilus influenzae*. IgG1 and IgG3 are responsible for the antibody response to protein antigens. The role of a selective IgG4 deficiency as a risk factor for recurrent infections is unknown, since it occurs in many apparently normal individuals. Additionally, an accurate detection of very low levels of IgG4 is technically difficult to achieve, therefore a selective deficiency of IgG4 alone is difficult to interpret.[8] An IgG subclass deficiency gains clinical significance if it is associated with recurrent infections and/or a defect in antibody responsiveness.

The aim of this retrospective study was to review the clinical findings and laboratory results of 59 patients with IgG subclass deficiencies in order to determine their changes in serum IgG subclass levels during follow-up and to evaluate the percentage and time span until normalization of their IgG subclass levels to age-related normal levels, the type of infections occurring during the deficiency period, the type of prophylaxis and the benefits of prophylactic therapy in the study group.

MATERIALS AND METHODS

The case records of 59 pediatric patients (25 girls [42%], 34 boys [58%]) with IgG subclass deficiencies who had been referred to the Pediatric Immunology Department at Ege University's Children Hospital from January 1997 to May 2008 were reviewed. An IgG subclass deficiency was defined as one or more abnormal serum IgG subclass levels (more than 2 SD below the normal mean for age) with relevant symptoms.[2] Patients with an IgG1 subclass deficiency were not considered because they were evaluated within the perspective of hypogammaglobulinemia. Recurrent infections were defined as the presence of at least six febrile episodes requiring antibiotic treatment in one year. Patients affected by severe combined immunodeficiencies, common variable immunodeficiencies, hyper IgM syndromes or acquired immunodeficiency syndrome were not included in this study.

The quantifications of serum IgA, IgM, IgG and IgG1, IgG2, IgG3 had been performed by the nephelometric method with a Dade Behring BN2 nephelometer analyzer and commercially available kits by Dade Behring (Germany). Enzyme immunoassays had been used for the determination of IgG-antibodies against polyribosylribitol-phosphate of *Haemophilus influenzae* type B (Hib) and anti-tetanus toxoid antibodies (Immunozyme HiB IgG and Immunozyme Tetanus, Progen Biotechnik, Heidelberg, Germany). Protective titers for anti-Hib antibodies were defined as > 1 μg/ml and protective titers for anti-tetanus antibodies were defined as > 100 mIU/ml.[9,10]

Patients who had less than six mild infections during follow-up were not given a prophylactic therapy. Patients who had 7-10 mild upper respiratory tract infections such as otitis media, sinusitis or urinary tract infections requiring antibiotic treatment in one year were given an antibiotic prophylaxis (benzathine penicillin once every 21 days or an oral antibiotic such as amoxycillin, 25% of the total daily dose every night). Patients with more than 10 mild infections received a bacterial lysate prophylaxis (OM-85-BV-Bronchovaxom, Switzerland). Intravenous immunoglobulin (0.5 g/kg) was given to the patients who needed at least two hospitalizations in one year because of severe infections, such as pneumonia, tracheobronchitis, etc.

The records of all patients were evaluated for the following criteria: initial age at the time of infections, localization and number of infections, atopy (the diagnosis of atopy was derived from clinical findings such as recurrent eczema, bronchiolitis and/or allergic rhinitis, and laboratory examinations such as serum IgE levels, specific IgE levels and food panels when indicated), history of adenoidectomy/tonsillectomy, type, duration and efficacy of prophylactic therapy against infections, serum concentrations of IgM, IgG, IgA, IgG subclasses during follow-up (every three or six months) and their com-

parison to age-related normal levels, specific antibody responses against tetanus and *H. influenzae* type B, age at normalization of the relevant IgG subclass and percentage of the patients whose decreased levels of immunoglobulins reached normal levels. Specific antibody responses were obtained on admission and two months after vaccine application. In the case of IVIG replacement therapy, antibody responses were obtained before and again 2 months after Ig administration.

This study was approved by the Ethics Committee of the Faculty of Medicine of Ege University.

Statistical analyses

The data were analyzed using $\chi 2$, Kruskal-Wallis and Mann Whitney U tests (SPSS 12 for Windows). *P*-values less than 0.05 were considered statistically significant.

RESULTS

The mean age at the diagnosis of the IgG subclass deficiency was 4.20 ± 2.62 years. The mean age at the onset of recurrent infections was 2.36 ± 2.02 and the mean duration until diagnosis was 1.94 ± 1.95 years. Among the 59 patients studied, the most frequent defect was an IgG3 subclass deficiency (77%, n = 46). Nine percent (n = 5) of the patients had an isolated IgG2 deficiency and 14% (n = 8) had an IgG2+G3 deficiency.

The most common clinical presentations were recurrent upper respiratory tract infections (tonsillitis/pharyngitis) (63%), followed by upper respiratory tract infections combined with pneumonia (22%), upper respiratory tract infections combined with acute gastroenteritis (7%), upper respiratory and urinary tract infections (5%), and others (eczema, recurrent conjunctivitis) (3.4%). Atopy was found in 15% (n = 9) of the patients studied (bronchial hyperreactivity, n = 4; allergic rhinitis, n = 3; atopic dermatitis, n = 2). No significant association was observed between a specific subclass deficiency and the type of infection acquired (respiratory tract infections, urinary tract infections or gastroenteritis) (p = 0.05).

The mean levels of immunoglobulins at the time of diagnosis were as follows: IgM 108.7 ± 51.1 mg/dl, IgG 776.7 ± 256.7 mg/dl, IgA 77.4 ± 51.0 mg/dl, IgG1 482.5 ±261.1, IgG2 173.7 ± 207.8 mg/dl, IgG3 139.4 ± 78.6 mg/dl (Table 1). An anti-Hib antibody response was determined in 43% of the patients (n = 25). Preventive antibody titers (> 1 μg/ml) against this polysaccharide antigen were found in 38% of the patients (n = 22). An anti-tetanus antibody response was determined in 49% (n = 29) of the patients and 31% (n = 18) had a normal protective response (> 100 mIU/ml). There was no significant difference between IgG1, IgG2 and IgG3 subclass deficiency and the specific antibody responses (p = 0.152 and p = 0.826, respectively, for anti-tetanus and anti-Hib).

Fifty-three patients (90%) were given a prophylactic treatment to prevent infections. The mean age of the children during the first prophylactic ther-

Table 1 Immunoglobulin levels at diagnosis (A) and during follow-up at the last visit (B)

	A **Age: 4.20 ± 2.62 years** (mg/dl)	**B** **Age: 6.28 ± 1.36 years** (mg/dl)
IgM	108.7 ± 51.1	102.6 ± 33.8
IgG	776.7 ± 256.7	865.8 ± 209.2
IgA	77.4 ± 51.0	96.6 ± 54.4
IgG1	482.5 ± 261.1	462.6 ± 289.9
IgG2	173.7 ± 207.8	240.2 ± 254.1
IgG3	139.4 ± 78.6	185 ± 116.5

apy was 4.4 ± 2.2 years, and the mean duration of the prophylaxis was 13.5 ± 10.5 months. The mean durations of prophylaxis in respect to the study groups are given in Table 2. Antibiotic prophylaxis was administered to 42% (n = 25) of the patients. Oral bacterial lysate and intravenous immunoglobulin were given to 33% and 15% of the patients, respectively. Ten percent of the patients received no prophylactic treatment. The frequency of recurrent infections decreased from 13.4 ± 7.4 per year to 5.7 ± 3.9 in all 53 patients receiving a prophylactic regimen. The decreases in the frequency of infections for the different IgG subclass deficiency groups are shown in Table 2. There were no significant differences between frequency of infections and type of subclass deficiency or the prophylaxis chosen (p = 0.975 and p = 6.733, respectively). Five percent (n = 3) of the patients underwent adenotonsillectomy.

Serum IgG subclass levels reached normal ranges for their age in 30% of the patients of the IgG3 deficiency group and in 35.7% of the patients of the IgG2+G3 deficiency group. During follow-up, none of the serum IgG2 measurements in patients with isolated IgG2 deficiency had risen to age-related normal levels. The mean age of normalization of IgG subclass levels for these patients was 6.00 ± 1.73 years for IgG2 and 6.50 ± 0.58 years for IgG3 (Table 2).

DISCUSSION

Over the past two decades, IgG subclass deficiency has become a popular explanation for apparent infection-susceptibility in children and adults.[11] If the total immunoglobulin concentration is normal and the child's history warrants further evaluation, serum IgG subclass levels and specific antibodies against common antigens should be determined.

Recurrent infection was the most common presentation of an IgG subclass deficiency in our study as well as in most previous studies. The most prevalent recurrent infections were recurrent upper respiratory tract infections (tonsillitis/pharyngitis). It is well documented in the literature that IgG subclass deficiency is associated with recurrent pneumonia, and bronchiectasis.[12-14] Nevertheless, none of our patients had findings consistent with chronic lung disease.

Chong *et al.*[15] reported that IgG2 deficiency (9/15 patients, 60%) was the most common IgG subclass deficiency in their cohort. Recurrent sinopulmonary infections accounted for the majority of infections while the rest were infections occurring at other sites including the urinary tract, eyes, gastrointestinal tract and cervical lymph nodes. In their study, three patients (15%) had autoimmune prob-

Table 2 Overall evaluation of the study groups with respect to the frequency of infections, duration of prophylaxis and normalization of IgG subclass levels

	Age at diagnosis (years)	**Frequency of recurrent infections at diagnosis** (per year)	**Frequency of recurrent infections after prophylaxis** (per year)	**Duration of prophylaxis (months)**	**Age at normalization of IgG subclass levels** (years)	**Duration of normalization**	**Percentage of patients with normalized levels**
IgG2 deficiency group	4.90 ± 3.57	10.60 ± 5.81	7.80 ± 9.17	18.00 ± 16.24	NA	NA	NA
IgG3 deficiency group	4.19 ± 2.64	13.86 ± 7.63	5.40 ± 2.97	11.66 ± 7.57	6.11 ± 1.76	2.16 ± 1.96	30.0
IgG2 + IgG3 deficiency group	4.13 ± 1.97	12.12 ± 6.81	5.85 ± 3.97	22.16 ± 18.03	6.00 ± 1.73 for IgG2 6.50 ± 0.58 for IgG3	1.90 ± 0.74 for IgG2 2.25 ± 0.64 for IgG3	35.7

NA: not applicable, because during follow-up none of the serum IgG2 measurements in patients with isolated IgG2 deficiency group had reached age-related normal levels.

lems such as juvenile idiopathic arthritis, Henoch Schönlein Purpura and juvenile dermatomyositis. None of our patients had such autoimmune problems.

Atopic manifestations were also common in our group of patients with IgG subclass deficiency (15%). In our previous study, atopy was present in 24% of the patients with IgA and/or IgG subclass deficiency.[6] In Chong *et al.*'s study[15] allergic manifestations were more common (40%) than in ours (15%).

IgG2 deficiency is reported to be the most prevalent IgG subclass deficiency in pediatric patients.[14,15] De Baets *et al.*[16] studied the incidence of IgG subclass deficiency in 53 children with recurrent bronchitis of whom 30 (57%) were deficient in one of the IgG subclasses. In their study, more than half of the cases were IgG4 deficient and the rest shared IgG2 and IgG3 deficiencies equally. Ozkan *et al.*[14] from a different center in our country, found that 8.4% of the patients with recurrent respiratory infections had an IgG subclass deficiency. In that study, 42.1% of the patients had IgG2, 45.4% had IgG3, and 10.5% had both IgG2 and IgG3 deficiencies. In our study of 59 patients, the most frequent defect was an IgG3 subclass deficiency (77%). Nine percent of the patients had an isolated IgG2 deficiency and 14% had a combined IgG2+G3 deficiency. In various studies investigating IgG subclass deficiencies, different ratios of recurrent sinopulmonary infections have been found. This variation can be attributed to epidemiological differences between the selected patient groups and whether IgG4 levels were taken into account or not. In our study, we did not include IgG4 abnormalities, because IgG4 levels vary widely in normal persons, and many entirely normal persons have no demonstrable IgG4 by standard techniques.[7]

Patients with an IgG subclass deficiency may have difficulties in producing specific antibody responses. In our previous study, the percentages of patients with IgA and/or IgG subclass deficiency who could not produce preventive amounts of antibodies against tetanus were 2% and 4% for *H. influenzae* type B.[6] In this study, vaccine failures stood at 5% for *H. influenzae* type B and 18% for tetanus, therefore children with IgG subclass deficiencies should be checked for their specific antibody production after vaccinations.

The management of patients with IgG subclass deficiencies is still being discussed. The causative bacteria in the majority of respiratory tract infections are *Streptococcus pneumoniae, H. influenzae* type B and *Moraxella catarrhalis*. Bacterial infections of the respiratory tract are often treated empirically; several protocols of antibiotic treatments have been proposed such as antibiotic prophylaxis and/or periodic antibiotic therapy. Surgical intervention in selected patients with recurrent otitis and sinusitis could be an alternative preventive therapy. IgG replacement therapy is usually considered when the patient shows evidence of chronic infections and immunodeficiency and fails to respond to other therapies.[17] In our study group, the frequency of recurrent infections decreased from 13.4 ± 7.4 per year to 5.7 ± 3.9 in patients receiving any of the prophylactic regimens. No significant difference was observed between the frequency of infections and the deficiency of a specific subclass or the type of prophylaxis preferred. However, administration of either antibiotics or oral bacterial extracts was useful for the management of these patients with recurrent infections, in order to decrease morbidity and school absenteeism. Five percent of our study group underwent adenotonsillectomy. Intravenous immunoglobulin was required in 15% of the patients receiving prophylaxis. Olinder-Nielsen *et al.*[18] examined the efficacy of immunoglobulin prophylaxis in 350 adult patients who had a combined or selective IgG subclass deficiency but no IgA deficiency and suffered from recurrent respiratory tract infections (RTI). The authors reported an overall reduction in the RTI frequency of 59% and 63%, for IgG2 and IgG3 deficiency, respectively.

It is known that IgG1 and IgG3 levels increase as children grow up and reach adult levels earlier than the IgG2 and IgG4 subclasses.[19] Shackelford *et al.*[20] studied 9 children with IgG2 subclass deficiency and normalization in IgG2 levels was noticed in 4 children during 1 to 5 years of follow-up. In our previous study, serum IgG subclasses increased to normal levels at about six years of age in 67% of patients with a partial IgA+IgG subclass deficiency.[6] In this study, serum IgG subclass levels increased to normal ranges for age in 30% of the pa-

tients in the IgG3 deficiency group and in 35.7% of the patients in the IgG2+G3 deficiency group at the ages of 6.00 ± 1.73 years for IgG2 and 6.50 ± 0.58 years for IgG3. No increase in serum IgG2 measurements was observed in patients with isolated IgG2 deficiency during follow-up.

In conclusion, pediatric patients with an IgG subclass deficiency commonly present with recurrent infections. Such children may benefit from appropriate prophylactic treatment such as vaccines, oral bacterial extracts, antibiotics or intravenous immunoglobulin to avoid complications of recurrent infections until full immunological maturation is achieved. The findings of our study suggest that although IgG subclass levels may normalize in 30 to 40% of the patients at about 6 years of age, most take longer to recover and in some patients like in those with isolated IgG2 deficiencies these low levels may never resolve. Therefore, we emphasize the need of monitoring IgG levels together with the clinical symptomatology in affected individuals and initiate preventive measures when appropriate.

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