Serum immunoglobulin(IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique

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Immunoglobulin (Ig) G, IgM, IgA and IgG subclass (IgG1, IgG2, IgG3, IgG4) concentrations were determined in more than 500 healthy Turkish children using nephelometric technique. These parameters were thought to be highly varied for different ethnic groups because of environmental and genetic factors. Methodology used in previous studies has been reported to affect age-related normal values. Serum IgG, IgM, IgA and IgG1, IgG2, IgG3, IgG4 levels were measured in 510, 491, 486, 542, 511, 515, and 545 healthy children, respectively. According to their age, the patients were divided into 14 groups. In contrast to most of the previous studies, age-related normal values for IgG4 levels were also obtained. In conclusion, it has been suggested that our study as an example for Caucasians using nephelometric technique will supply useful information about age-related normal serum immunoglobulin and IgG subclass concentrations.

Key words: age related normal values, immunoglobulin, IgG subclasses.

Immunoglobulin (IgG, IgA, IgM) and IgG subclass concentrations in the sera of healthy children in different age groups have been measured and reported in a limited number of studies and are thought to be highly varied between different ethnic groups because of environmental and genetic factors[1-4]. In addition, methodology is very important for accurate and sensitive measurements. In previous studies, numerous immunological methods, such as enzyme-linked immunosorbent assay (ELISA), radial immunodiffusion (RID) and immunonephelometry have been used to study the normal ranges and the role of the individual subclasses in humoral immune response[5-9].

For the diagnosis of primary immunodeficiencies, some autoimmune disorders, acquired immunodeficiency syndromes and various isolated clinical conditions, the clinicians need accurate age-related normal ranges of immunoglobulins and IgG subclasses for their own ethnic origin. Besides selective IgA deficiency, IgG subclass deficiencies are the most common form of humoral immunodeficiencies. Some patients may have deficiencies of one or more IgG subclasses with normal levels of total IgG, because IgG1, IgG2, IgG3, and IgG4 are found to be approximately 65%, 20%, 10% and 5% of the total serum IgG, respectively[10-13].

In this study, as an example of levels in Caucasians, normal values and age-related reference ranges for serum immunoglobulins and IgG subclass levels were determined by nephelometric measurements in more than 500 healthy Turkish children. Such a study, with a large series of children and using nephelometric technique, was as yet not available in this geographical area.

Material and Methods

Serum IgA, IgG, IgM and IgG1, IgG2, IgG3, IgG4 concentrations were determined using nephelometric technique in children visiting the well-baby clinic of Ege University, Department of Pediatrics. The patients admitted for minor trauma and surgery were also included. The study

was carried out prospectively. Children with a medical history of chronic disease, recurrent infections, atopy and immunodeficiency were not included. Age of the children varied from 1 day to 16 years. Serum IgG, IgM, IgA and IgG1, IgG2, IgG3, IgG4 levels were measured in 510, 491, 486, 542, 511, 515, and 545 healthy children, respectively. According to their age, the patients were divided into 14 groups as follows: group 1 (1 to 30 days), group 2 (1 to 5 months), group 3 (6 to 8 months), group 4 (9 to 12 months), group 5 (13 to 24 months), group 6 (25 to 36 months), group 7 (37 to 48 months), group 8 (49 to 72 months), group 9 (7 to 8 years), group 10 (9 to 10 years), group 11 (11 to 12 years), group 12 (13 to 14 years), group 13 (15 to 16 years), and group 14 (more than 16 years, adults).

Informed parental consent was obtained. Blood samples of participants were obtained by venipuncture and serum was separated by centrifugation. The samples were examined on the same day that the blood was taken.

The quantifications of serum IgA, IgG, IgM and IgG1, IgG2, IgG3, IgG4 were performed by nephelometric method on the Dade Behring BN2 Nephelometer Analyzer and commercially available kits by Dade Behring, Germany. The coefficients of intra-assay and inter-assay variations to immunoglobulins and IgG subclasses on the Behring Nephelometer were less than 2.7% and less than 4.4%, respectively. Serum immunoglobulins and the IgG subclasses were expressed as mg/dl. The results were statistically analyzed using SPSS software. Results are given as geometric mean, standard deviation, minimum and maximum ranges and 95% confidence intervals.

Results

Age-related geometric mean, standard deviation, minimum and maximum ranges and 95% confidence intervals for serum IgA, IgG, IgM and IgG1, IgG2, IgG3, IgG4 concentrations are listed in Tables I-VII.

Because of maternal IgG, serum IgG and IgG subclass levels were very high in the first group presenting in the neonatal period (0-30 days). In the second group with children between 1-5 months, these parameters reached the lowest level because of loss of maternal antibodies and inadequate synthesis by the infant. After Group 3 (6-8 months), all the results showed a gradual increase in the levels of different immunoglobulins and IgG subclasses with increasing age. As expected, IgA and IgM levels were very low in the neonatal period and then began to increase gradually. IgG, IgA, IgG1 and IgG2 levels increased until 9-10 years of age and then reached a plateau. IgG3 and IgG4 levels increased until 7-8 years of age and reached a plateau thereafter. IgM levels increased earlier and after the first year of life reached a stable value.

The point at which concentrations became similar to levels in adults (Group 14, subjects older than 16 years) was highly varied and these levels were not necessarily the highest values.

Table I. Age-Related Serum IgG Levels (mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	16	884.2 ± 230.4	492–1190	[792.0, 1037.5]
1-5 months	12	473.8 ± 193.1	270–792	[384.2, 629.7]
6-8 months	18	581.9 ± 207.9	268–898	[515.6, 722.4]
9-12 months	26	692.7 ± 181.1	421–1100	[641.9, 788.2]
13-24 months	60	774.4 ± 199.7	365–1200	[748.2, 851.4]
25-36 months	52	822.3 ± 208.4	430–1290	[790.4, 906.4]
37-48 months	40	879.9 ± 157.2	539–1200	[844.1, 944.6]
49-72 months	70	986.2 ± 209.6	528–1490	[958.5, 1058.5]
7-8 years	66	1040.7 ± 203.2	527–1590	[1011.5, 1111.4]
9-10 years	57	1062.8 ± 238.8	646–1620	[1024.9, 1151.7]
11-12 years	34	1051.7 ± 228.9	579–1610	[995.9, 1155.6]
13-14 years	25	1087.8 ± 236.0	741–1650	[1014.2, 1209.0]
15-16 years	17	981.1 ± 207.7	666–1370	[895.3, 1108.9]
Older than 16 years	17	1224.9 ± 280.2	830–1820	[1109.9, 1398.0]

Table II. Age-Related Serum IgA Levels (mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	16	5.7 ± 0.2	5.0–5.8	[5.6, 5.9]
1-5 months	12	20.2 ± 19.7	5.8–58.0	[15.8, 40.9]
6-8 months	15	23.2 ± 25.2	5.8–85.8	[20.5, 48.5]
9-12 months	26	52.9 ± 36.7	18.4–154.0	[47.2, 76.9]
13-24 months	57	44.1 ± 18.3	11.5–94.3	[42.9, 52.6]
25-36 months	52	53.5 ± 26.8	23.0–130.0	[51.4, 66.3]
37-48 months	39	68.8 ± 22.2	40.7–115.0	[64.8, 79.2]
49-72 months	68	91.9 ± 37.4	23.0–205.1	[90.2, 108.3]
7-8 years	64	108.4 ± 42.3	36.1–268.0	[105.9, 127.0]
9-10 years	53	116.7 ± 45.9	54.0–268.0	[111.8, 137.0]
11-12 years	31	115.8 ± 43.0	27.0–198.0	[109.7, 141.3]
13-14 years	23	130.5 ± 47.4	52.4–225.0	[118.0, 159.0]
15-16 years	15	109.8 ± 29.4	48.0–158.0	[97.8, 130.3]
Older than 16 years	15	121.3 ± 55.5	46.5–221.0	[102.4, 163.8]

Table III. Age-Related Serum IgM Levels (mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	14	18.5 ± 3.5	17.3–29.6	[16.7, 20.7]
1-5 months	11	57.3 ± 37.4	18.4–145.0	[41.9, 92.1]
6-8 months	17	68.7 ± 38.9	26.4–146.0	[58.5, 98.5]
9-12 months	27	86.1 ± 40.3	23.5–180.0	[78.9, 110.8]
13-24 months	57	98.3 ± 40.3	25.6–201.0	[96.3, 117.7]
25-36 months	53	92.5 ± 33.9	36.0–199.0	[89.0, 107.7]
37-48 months	38	86.1 ± 35.3	26.1–188.0	[80.9, 104.0]
49-72 months	69	105.8 ± 40.8	33.3–207.0	[103.7, 123.3]
7-8 years	65	97.6 ± 42.9	30.5–220.0	[95.5, 116.8]
9-10 years	53	93.9 ± 49.3	33.7–257.0	[90.8, 118.0]
11-12 years	32	102.4 ± 38.8	30.0–187.0	[96.0, 124.0]
13-14 years	24	120.9 ± 43.8	44.0–206.0	[110.3, 147.3]
15-16 years	15	99.7 ± 49.7	33.0–205.0	[83.7, 138.8]
Older than 16 years	16	130.9 ± 44.5	75.0–198.5	[114.6, 161.9]

Table IV. Age-Related Serum IgG1 Levels (mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	16	675 ± 152	430–897	[611, 773]
1-5 months	11	319 ± 113	160–574	[261, 413]
6-8 months	14	485 ± 188	279–820	[408, 625]
9-12 months	29	562 ± 240	328–1250	[506, 690]
13-24 months	67	721 ± 292	344–1435	[702, 844]
25-36 months	60	736 ± 285	340–1470	[712, 860]
37-48 months	49	762 ± 246	439–1333	[726, 867]
49-72 months	58	755 ± 209	468–1333	[726, 837]
7-8 years	63	806 ± 281	420–1470	[778, 920]
9-10 years	66	860 ± 329	380–1840	[834, 996]
11-12 years	35	842 ± 241	599–1560	[787, 953]
13-14 years	32	872 ± 354	490–1560	[805, 1061]
15-16 years	21	796 ± 269	498–1460	[711, 956]
Older than 16 years	21	857 ± 214	528–1384	[782, 978]

Table V. Age-Related Serum IgG2 Levels(mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	15	156 ± 50	87–263	[135, 192]
1-5 months	10	59 ± 26	32–108	[46, 84]
6-8 months	14	67 ± 37	36–146	[53, 97]
9-12 months	29	64 ± 35	25–161	[58, 85]
13-24 months	67	93 ± 49	31–264	[92, 116]
25-36 months	58	115 ± 85	43–380	[112, 157]
37-48 months	44	161 ± 92	60–410	[155, 211]
49-72 months	52	167 ± 78	85–440	[160, 204]
7-8 years	60	197 ± 101	67–460	[193, 245]
9-10 years	62	214 ± 121	70–543	[211, 273]
11-12 years	32	212 ± 88	111–515	[195, 259]
13-14 years	28	279 ± 134	100–573	[257, 361]
15-16 years	20	238 ± 83	110–398	[214, 292]
Older than 16 years	20	307 ± 128	147–610	[271, 391]

Table VI. Age-Related Serum IgG3 Levels(mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	15	37 ± 17	18–78	[31, 50]
1-5 months	10	24 ± 12	13–53	[17, 35]
6-8 months	14	35 ± 25	14–100	[27, 56]
9-12 months	29	38 ± 24	18–110	[34, 53]
13-24 months	65	37 ± 25	16–132	[37, 49]
25-36 months	54	32 ± 21	14–125	[30, 42]
37-48 months	45	37 ± 25	15–120	[35, 50]
49-72 months	54	37 ± 20	15–107	[36, 47]
7-8 years	62	51 ± 43	21–186	[51, 73]
9-10 years	65	51 ± 34	20–186	[50, 67]
11-12 years	34	53 ± 40	29–200	[47, 75]
13-14 years	29	80 ± 56	28–223	[73, 117]
15-16 years	18	58 ± 21	30–120	[51, 73]
Older than 16 years	21	50 ± 33	21–152	[43, 73]

Table VII. Age-Related Serum IgG4 Levels(mg/dl) in Healthy Children

Age groups	Number of subjects	Geometric mean ± SD	Min-max	95% confidence intervals
0-30 days	15	24 ± 17	17–81	[17, 36]
1-5 months	10	15 ± 14	2–48	[10, 31]
6-8 months	15	14 ± 11	2–52	[12, 25]
9-12 months	27	12 ± 5	2–20	[12, 16]
13-24 months	69	16 ± 17	2–99	[18, 26]
25-36 months	61	20 ± 40	2–171	[23, 43]
37-48 months	50	27 ± 37	4–185	[27, 48]
49-72 months	56	35 ± 46	8–227	[37, 62]
7-8 years	64	42 ± 46	2–198	[49, 72]
9-10 years	69	36 ± 45	5–202	[41, 63]
11-12 years	35	34 ± 44	4–160	[34, 64]
13-14 years	31	51 ± 45	10–144	[51, 84]
15-16 years	20	36 ± 44	9–187	[30, 72]
Older than 16 years	23	33 ± 47	15–202	[25, 66]

Discussion

As new groups of immunodeficiencies are defined and potential therapies advocated, careful attention should be given to establishing the age-specific normal limits of serum immunoglobulins and IgG subclasses. The discordant results obtained in previous studies are thought to be due to methodology[14-17].

In recent years, different methods for quantitative determination of serum immunoglobulins and IgG subclasses have been developed. ELISA with specific monoclonal antibodies, RID using polyclonal or monoclonal antibodies and nephelometric assays have been used by various investigators. There is still no agreement on the most sensitive and accurate method[18-22]. However, with RID method, results are obtained after an incubation period of 72 hours. In the last few years, nephelometry has begun to be widely used. Nephelometric assays allow determination of serum immunoglobulins and IgG subclass concentrations in a large number of samples easily, rapidly (less than 15 minutes for a complete immunoglobulin and IgG subclass profile), reproducibly (intra-assay variation 2.1-2.7%, inter-assay variation 1.9-4.4%) and precisely[16,23,24]. Therefore, we used nephelometric method to determine serum immunoglobulins and IgG subclasses in this study.

Genetic, ethnic and geographical differences are also very important in determining normal ranges for serum immunoglobulins. Ambrosino et al.[3] reported that young black children have lower IgG1, IgG2 and IgG4 serum concentrations than found in white children and no consistent differences were noted for IgG3 subclass values. In French and Harrison's study[25] performed in the United Kingdom, IgG subclass concentrations in adults were similar to those in European populations. In our study, as an example of Caucasian values, normal values and age-related reference ranges for serum immunoglobulins and IgG subclass levels were determined by nephelometric measurements in healthy Turkish children. From our country, Berkel et al.[26] studied and reported IgG1, IgG2, IgG3 values in 353 healthy children and adults using RID method. Despite good correlation between IgG1 and G2 levels, our study showed lower mean levels and ranges of IgG3 than those obtained by Berkel et al.[26]. Differences between these two studies might be related to laboratory techniques. Vlug et al.[16] also studied IgG subclasses by nephelometry in the Netherlands and their IgG3 levels are in good correlation with our findings.

IgG4 deficiency can be an isolated phenomenon or it can occur in combination with deficiencies of IgG2 and/or IgA. Isolated IgG4 deficiency is associated with symptoms and signs which are similar to those associated with other IgG subclass deficiencies[27]. In most of the previous studies, the broad spread of IgG4 values in children did not permit calculation of reference values[8,28]. In Berkel et al.'s study[26], IgG4 levels showed a non-homogeneous distribution until the age of 16 years and normal ranges for IgG4 could not be established and reported. However, using nephelometric technique, we were able to calculate age-related geometric mean, standard deviation, minimum and maximum ranges and 95% confidence intervals for serum IgG4.

In most of the previous studies, no significant differences in serum immunoglobulins and IgG subclasses between male and female subjects were found in each of the age groups studied[29], so we did not analyze nor include data related with sex in the Tables.

Serum IgG level was found to be high in the neonatal period because IgG is known to be the only immunoglobulin that can cross the placenta. Over the first 4-5 months of life, there was a gradual decrease in the serum IgG levels and a gradual increase in the serum IgM and IgA levels. Then, IgG levels began to increase with age and reached a plateau by the age of 9-10 years. In the literature, it has been reported that IgM, IgG and IgA levels reach those of adult values by one year of age, 6-8 years and 10 years, respectively[26,27,29,30]. Results of our study showed similar trends in immunoglobulin levels. However, in contrast to some of the previous studies, our IgM levels reached a stable value a few months earlier and higher IgM levels were found throughout the different age groups. It has been thought that this might be due to more exposure to infectious agents and antigens in childhood in our population.

It has been reported that serum IgG1 and IgG3 concentrations reached adult values earlier than IgG2 and IgG4 levels[26,31]. However, in our study, IgG3 and IgG4 levels reached a plateau by the age of 7-8 years and thereafter IgG1 and IgG2 levels by the age of 9-10 years.

In conclusion, it has been suggested that our study using nephelometric technique will provide useful information about age-related normal serum immunoglobulins and IgG subclass concentrations for our country and also for some European countries.

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