The Turkish Journal of Pediatrics 36: 197-204 1994

SERUM IMMUNOGLOBULIN G SUBCLASS VALUES IN HEALTHY TURKISH CHILDREN AND ADULTS*

*A. İzzet Berkel MD**, İlhan Tezcan MD***, Fügen Ersoy MD** Özden Sanal MD***

SUMMARY: Berkel Aİ, Tezcan İ, Ersoy F, Sanal Ö. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum immunoglobulin G subclass values in healthy Turkish children and adults. Turk J Pediatr 1994; 36: 197-204.

Immunoglobulin G (IgG) subclass levels were determined in 393 serum samples of 329 healthy children from birth to 16 years of age, 24 healthy adults and 20 pairs of mother-cord blood by radial immunodiffusion. Age-normal percentile values for IgG1, IgG2 and IgG3 were calculated for age-groups up to 16 years and for adults. The broad range of IgG4 values in children did not permit calculation of reference values. *Key words: IgG subclasses, Immunoglobulins.*

Immunoglobulin G (IgG) subclass deficiencies have been associated with a variety of disorders including primary immunodeficiencies, recurrent respiratory tract infections and pyogenic infections, allergic disorders, autoimmune disorders, intractable epilepsy, acquired immunodeficiency syndrome and various isolated clinical conditions[1-8]. Deficiencies of one or more IgG subclasses with normal levels of serum IgG, IgM and IgA have been defined in children with recurrent infections, and some have been treated successfully with intravenous immunoglobulins[6,9,10]. IgG subclass deficiencies are the most common of humoral immunodeficiencies[11]. In recent years, since the availability of monoclonal antisera, the measurement of IgG subclasses has been included in diagnostic tests in clinical medicine. However, use of different laboratory techniques, variability of age-normal values and differences in IgG subclass values among populations have been the main concerns of physicians interpreting the results. Therefore, determination of age-normal values for different ethnic groups is considered mandatory in the evaluation of IgG subclass deficiencies.

In this study, IgG subclass levels were determined by radial immunodiffusion in healthy adults, pairs of mother and cord serum, and healthy children from birth to 16 years of age in order to establish age-normal reference values. The data obtained were used to construct age-normal percentile charts.

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* From the Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

*** Associate Professor of Pediatrics, Hacettepe University Faculty of Medicine.

Material and Methods

Serum specimens from 329 clinically healthy children (176 males, 153 females) from birth to 16 years of age with histories negative for allergy, recurrent infections and immunodeficiency in the family were examined. The patients had either been admitted to the hospital for minor surgery or had attended local health clinics. Informed consent was obtained from the parents. Twenty-four healthy adults (medical students or lab technicians) ranging in age from 18 to 34 years, the sera from 20 mothers and the cord blood of their babies were also included in the study.

Peripheral venous blood was drawn; serum was separated by centrifugation and then stored in aliquots at −20°C until use.

IgG subclass levels were determined by radial immunodiffusion using commercial kits from Binding Site Limited (University of Birmingham Research Institute, Birmingham, England). Samples to be tested were diluted 1:10 for IgG1 and IgG2; undiluted serum was used for IgG3 and IgG4. Plates were left at room temperature (22°C) for 72 hours and then read with an RID reader which can measure 0.1 mm fractions. Results were interpolated from a standard curve constructed from a pool of normal sera calibrated against WHO reference serum (67/97). When necessary, the subclass determination was repeated using low-level plates.

Total IgG, IgM and IgA levels were measured by turbitometry using the Behring Turbitimer (Behringwerke AG, Marburg, Germany). Only sera with normal levels of IgG, IgM and IgA for age were included in this study.

Statistical analysis: different statistical approaches were used to analyze the data of the children and adults.

For children, the second-order polynomial equation method was applied to the log 10 transformed data[12]. This method places the data on an age-normal subclass level curve excluding the first two months. Mean and ±3 SD curves were constructed for IgG1, IgG2 and IgG3. IgG4 values were not included because of the wide variations observed.

In the adults' data, there was no variation with age for any of the four subclasses.

Results

The numbers included in each age-group, including geometric means and ranges of the IgG1, IgG2 and IgG3 levels, are reported in Table I. Individual data, mean and ±3 SD curves for the three IgG subclasses are shown in Fig. 1. IgG subclass values for mothers and cord blood are shown in Fig. 2. Serum IgG1, IgG2 and IgG3 levels all tended to increase with age. However, the pattern of the curves differed for each subclass.

Table I: Serum IgG Subclass Values (g/L)
(Ranges for the Age-Groups are Given in Parenthesis)

Age	Number of Subjects	IgG1	IgG2	IgG3
0-30 days	12	9.25 (7.42-12.60)	1.83 (0.80-2.93)	0.57 (0.42-0.75)
31-60 days	12	3.05 (1.85-4.21)	1.11 (0.45-2.16)	0.32 (0.21-0.53)
3-5 months	12	3.42 (2.20-7.15)	0.62 (0.40-1.40)	0.44 (0.17-0.80)
6-8 months	12	3.81 (1.51-7.69)	0.62 (0.25-1.20)	0.48 (0.20-1.44)
9-12 months	16	4.02 (2.44-5.50)	0.46 (0.19-0.90)	0.46 (0.28-0.56)
13-24 months	23	6.45 (4.66-11.30)	0.93 (0.40-1.70)	0.60 (0.33-1.01)
25-36 months	24	7.16 (4.50-11.10)	1.10 (0.43-2.41)	0.55 (0.37-1.20)
37-48 months	24	7.97 (5.13-15.50)	1.37 (0.43-2.70)	0.60 (0.26-1.14)
49-72 months	40	7.40 (4.21-13.60)	1.61 (0.80-3.60)	0.56 (0.21-1.14)
7-8 years	40	7.68 (4.10-11.80)	1.91 (0.80-5.70)	0.78 (0.33-1.83)
9-10 years	30	8.81 (5.13-19.80)	2.38 (1.40-4.30)	0.86 (0.37-1.53)
11-12 years	24	10.26 (6.36-19.40)	2.16 (0.95-3.30)	0.89 (0.26-2.07)
13-14 years	30	8.00 (4.66-14.00)	2.39 (1.00-5.20)	0.86 (0.49-2.00)
15-16 years	30	8.98 (5.90-14.00)	2.33 (0.77-5.20)	0.93 (0.36-2.00)
Adults	24	.6.62 (4.10-12.30)	3.77 (2.16-5.90)	0.70 (0.16-1.38)
Cord serum	20	10.12 (6.10-14.00)	2.53 (1.26-4.39)	0.91 (0.39-1.70)
Mothers' serum	20	7.10 (4.66-9.72)	2.83 (1.26-6.26)	1.05 (0.58-1.82)

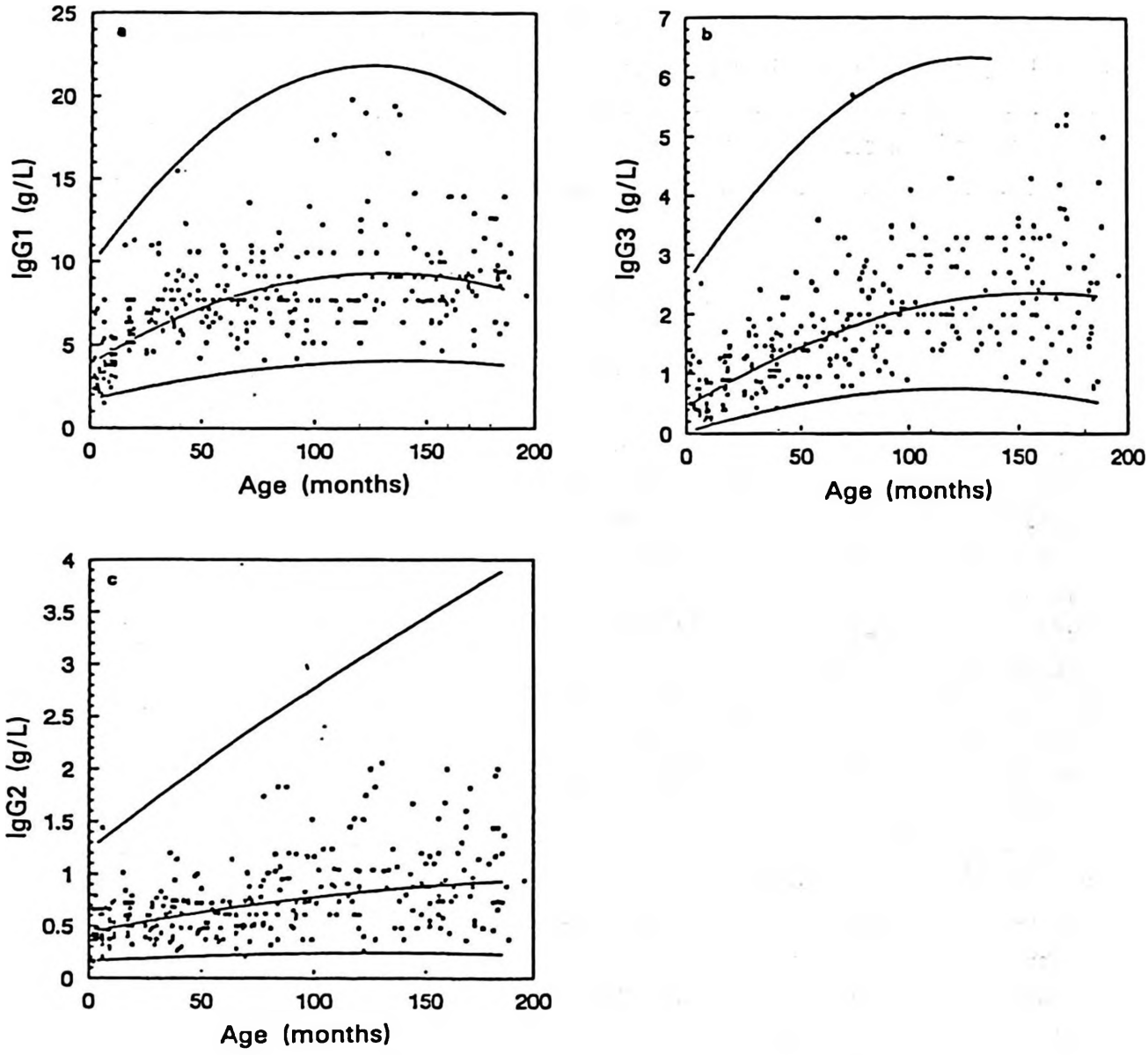

Fig. 1: Scatter diagrams of IgG subclass levels (Mean and ± 3 SD curves)
a) IgG1. b) IgG2. c) IgG3.

IgG1 levels increased gradually, peaked at approximately 12 years of age and decreased slightly thereafter. The values for adolescents were higher than those for adults. IgG1 levels in cord sera were higher than those of the mothers. IgG2 levels also increased with age, peaking at ten years of age, but reached only 67 percent of the adult levels at the age of 16. IgG2 levels of cord sera were the same or slightly lower than the mothers' serum levels. IgG3 levels increased until 12 years of age and reached a plateau thereafter. Adults had lower IgG3 levels than did adolescents. IgG3 levels were similar in cord and mothers' sera. IgG4 levels showed a non-homogeneous distribution until the age of 16 years;

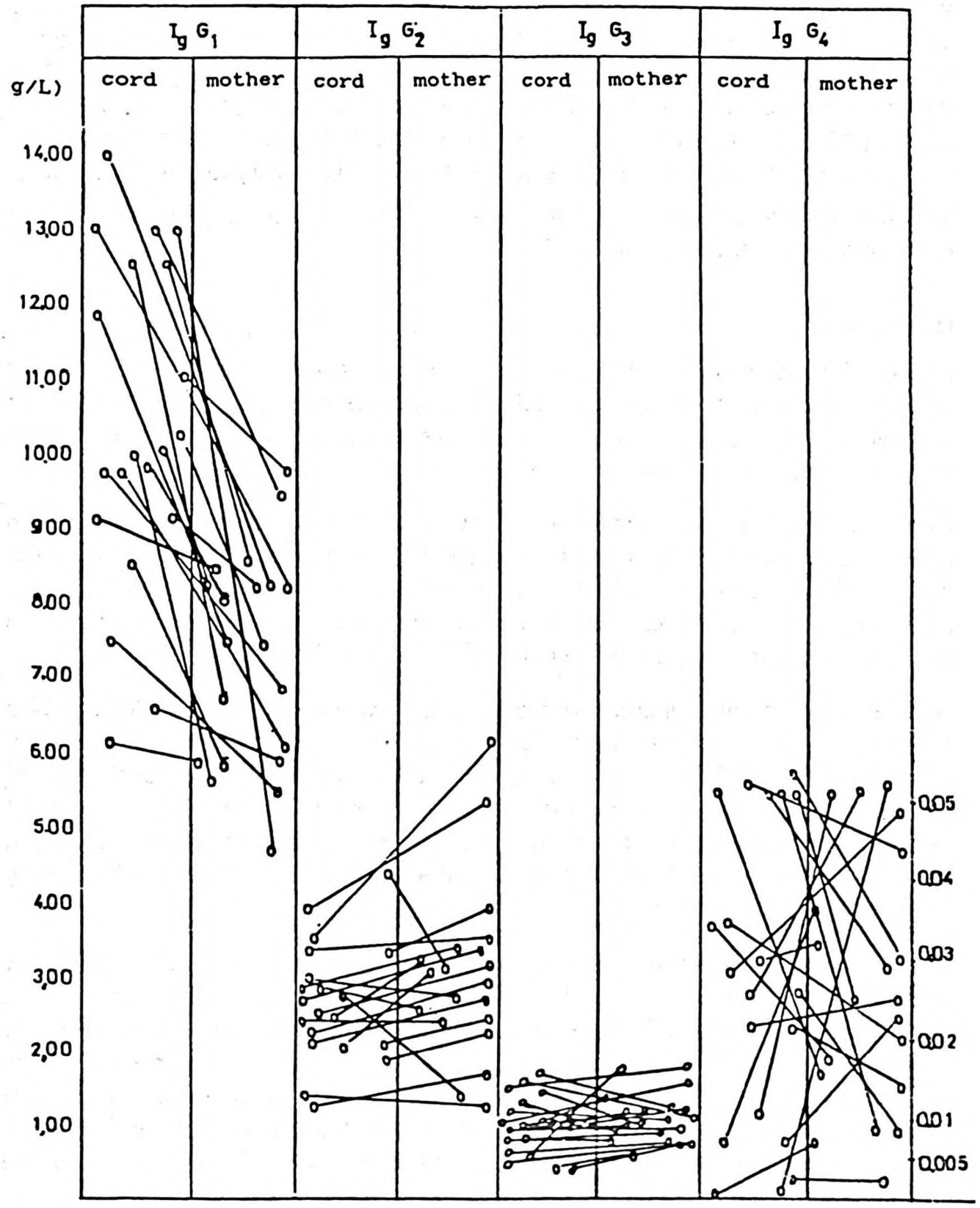

Fig. 2: IgG subclass values in cord and mother sera.

therefore, normal ranges for IgG4 could not be established. IgG4 values were very low (undetectable or <0.005 g/L) in 38 percent, greater than 0.05 g/L (above the upper reading range of IgG4 plate) in 24 percent, and between 0.005-0.05 g/L in the remaining 38 percent of tested sera. Twenty percent of adults had very low values (<0.005 g/L). In half of the cord sera, the IgG4 levels were higher than those of the mother, and in three samples the level was very low (<0.005 g/L).

The sum of the concentrations of the four subclasses corresponded with the concentration of total IgG (r: 0.7131).

Discussion

IgG subclass levels in healthy children and adults have been studied by various investigators. In some studies monoclonal antibodies were used[13-17], while other studies utilized polyclonal antisera[18-22]. In this study, IgG1, IgG2 and IgG3 levels were assessed using polyclonal antibodies.

During early childhood, serum IgG1 and IgG3 concentrations are the first to reach adult levels, with IgG2 and IgG4 maturation lagging behind[17,19,20]. We found higher IgG1, IgG2 and IgG3 (mean and-3 SD) values than were reported previously, except for IgG2 levels which were lower than many reported series in patients under the age of one year[7,9,15,17,19,20,23,24].

All IgG subclass levels increased progressively with age. IgG1 and IgG3 levels peaked at 12 years of age, while IgG2 peaked at approximately 16 years of age. However, IgG2 levels were approximately two-thirds of adult levels at the age of 16 years. IgG4 levels varied so widely that age-normal reference ranges could not be established. This finding is in accord with other studies where IgG4 levels were low in 10-20 percent of healthy adults and 8-21 percent of healthy children[14,16,17,19].

The mechanisms that regulate IgG subclass ontogeny are not completely understood. Regulation of IgG subclass concentrations may occur at many levels, such as B-cell subsets, T-cell subsets, lymphokine production or lymphokine receptor expression[13]. The correlation of IgG subclass levels with Gm factors supports the role of genetic factors[23,25,26]. Other potential factors for IgG subclass levels are differences in socioeconomic status, nutrition and environmental exposures in different populations or ethnic groups[13]. Constant exposure to antigens may result in higher IgG subclass levels[13,27]. We feel that the higher IgG1, IgG2 and IgG3 levels observed in our study may be a result of early or constant exposure to antigens in our society. The results of our study show that normal values are widely scattered in childhood, and the use of well-defined age-normal limits is crucial for the correct definition of true subclass deficiencies.

Acknowledgement

The authors wish to thank to Dr. Levent Öner of the statistical analysis. This work was supported by a grant (TAG 0752) from the Scientific and Technical Research Council (TÜBİTAK) of Turkey.

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