

Age patterns of immunoglobulins G, A & M in healthy children and the influence of breast feeding and vaccination status

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Serum levels of immunoglobulins G, A and M were determined by nephelometry in 414 infants and children aged 1 month to 14 years. The children were admitted to "Aghia Sophia" Teaching Hospital for Children for surgical corrections of minor anatomical abnormalities, but they were otherwise healthy. Statistical analysis was performed through multiple regression after logarithmic transformation of the immunoglobulin values. Immunoglobulin G (IgG) levels increased significantly by 18% per year until the age of 5 years and by 2% per year thereafter. Among children less than 5 years old, IgG levels tended to increase significantly with the number of doses of either DTP or Sabin vaccine. Immunoglobulin A (IgA) levels increased significantly by 27% until the age of 7 years and by 4% thereafter. Among children less than 7 years old, history of breast feeding was significantly associated with higher levels of IgA. Vaccination with either DTP or Sabin was associated with elevation of IgA levels among younger and, to a lesser extent, older children, but the elevations did not reach statistical significance. Immunoglobulin M (IgM) levels increased rapidly during the first 12 months of life and very slowly thereafter and they were significantly higher among girls. Among children less than one year old, there was evidence that vaccination with either DTP or Sabin was associated with elevated IgM levels, although the differences were not statistically significant possibly because of small numbers of infants in the study sample. The results of the present study demonstrate the age- and sex-dependent changes of immunoglobulins G, A and M, they indicate that vaccination is associated with higher immunoglobulin levels among younger children, and suggest that breast feeding may increase IgA levels among children less than 7 years old.

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Serum levels of immunoglobulins G, A and M (IgG, IgA, IgM) depend on a variety of genetic, developmental and environmental factors such as ethnic background, sex, age and history of infections (1–11). Age is especially important in the interpretation of immunoglobulin levels. Normal infants are born with very low levels of self-generated serum immunoglobulins; the entire IgG portion of cord serum has been transferred transplacentally from the mother. If an infection occurs in utero, cord IgM and IgA levels are elevated. After birth, maternal IgG decays and the levels of serum IgG are gradually decreasing; this trend is reversed with the onset of autologous IgG synthesis (12). Adult IgG, IgA and

IgM levels are reached at different ages in different population groups, but usually before late adolescence. Immunoglobulin levels are clinically useful in the diagnosis of patients with unusual susceptibility to infections, as well as with allergic or autoimmune diseases (4, 9, 10). We have studied serum IgG, IgA and IgM levels in Greek children who did not suffer from diseases or conditions that are believed to affect immunoglobulin status. The purpose of the study was to determine the age dependent patterns of immunoglobulin increase in the Greek paediatric population and to explore whether socio-demographic variables, vaccination status and history of breast feeding affect these patterns.

Table 1. Distribution of the 414 children by study variables.

Variable	Category or unit	Number	Percent (%)
Age	-5 months	20	4.8
	6-11 months	16	3.9
	1-2 years	89	21.5
	3-4 years	86	20.8
	5-6 years	63	15.2
	7-8 years	48	11.6
	9-10 years	46	11.1
	11-12 years	35	8.5
	13+	11	2.6
Sex	male	362	87.4
	female	52	12.6
Breast feeding*	none	121	31.6
	-3 months	183	47.8
	4-6 months	51	13.3
	>6 months	28	7.3
History of previous infections	positive	128	30.9
	negative	286	69.1
DTP vaccine*	-3 doses	196	50.4
	4+ doses	193	49.6
Sabin vaccine*	-3 doses	173	44.5
	4+ doses	216	55.5

* There were missing values for breast-feeding (31), DTP (25) and Sabin vaccine (25).

Material and method

Study population

Four hundred and fourteen infants and children (362 boys and 52 girls) aged 1 month to 14 years, consecutively admitted at the First and Second Surgical Departments in "Aghia Sophia" Teaching Hospital for Children for minor surgical operations from January to December 1992 were included in this study. All children were free from infection at the time of enrollment. More specifically 32% were admitted for inguinal hernia, 19% for cryptorchidism, 15% for phimosis, 13% for hydrocele, 9% for hypospa-

dias, 5% for umbilical hernia and varicocele and 7% for other anatomical abnormalities. A standardized interview was conducted by one of us (ML) with the parents of each child and informed consent was obtained. The questionnaire covered sociodemographic and medical history variables, namely age, sex, birth weight, breast feeding history, paternal occupation, previous health history and vaccination status with regard to DTP and Sabin vaccines. At the time of blood drawing for the determination of immunoglobulins, none of the children was taking any medications which could affect immune homeostasis and they were all free from infection. Individuals were divided into nine age groups: 1 to 5 months, 6 to 11 months, 1 to 2 years, 3 to 4 years, 5 to 6 years, 7 to 8 years, 9 to 10 years, 11 to 12 years, and 13 to 14 years.

In order to determine the serum levels of immunoglobulins, blood samples were centrifuged and the sera obtained were stored at -70°C . Serum immunoglobulin concentrations were quantified by nephelometry. Subjects' sera were diluted with isotonic saline 1:400 for IgG and 1:5 for IgA and IgM. A Behring nephelometer was used (Behringwerke AG, Marburg, Germany). Data were expressed in g^{-2}/l .

Statistical methods

The statistical analysis was undertaken after log-transformation of individual immunoglobulin values, because the frequency distribution of these values was positively skewed. The log-transformed values were then used in turn as dependent variables in multiple regression models with age, gender, birth weight, breast feeding, paternal occupation (manual, non-manual), previous health status (no health problems, history of frequent infections) as independent (predictor) variables simultaneously introduced, whereas vaccination status variables (number of doses) with respect to DTP and Sabin vaccines were alternatively introduced to avoid collinearity prob-

Table 2. Geometric means and normal range ($\pm\text{SD}$)* of IgG, IgA and IgM (in g^{-2}/l) by age in children

Age	N	IgG		IgA		IgM	
		Geometric mean	Normal range	Geometric mean	Normal range	Geometric mean	Normal range
1 to 5 months	20	490	224-1072	22	6-81	65	31-135
6 to 11 months	16	603	316-1148	30	13-69	98	47-204
1 to 2 years	89	912	575-1446	54	23-123	126	63-251
3 to 4 years	86	1072	708-1622	89	32-245	132	69-251
5 to 6 years	63	1202	759-1905	107	34-339	138	65-282
7 to 8 years	48	1175	812-1698	117	65-214	141	62-324
9 to 10 years	46	1349	891-2042	132	52-331	132	63-275
11 to 12 years	35	1349	977-1862	151	69-331	151	76-302
13 to 14 years	11	1380	955-1995	135	85-214	155	65-372

* after log-transformation and, eventually, inversion.

Table 3. Multiple regression-derived proportional changes of IgG, IgA and IgM levels among the respective younger age groups by indicated changes in a series of predictor variables

Age group Predictor Variable	Less than 5 years old		Less than 7 years old		Less than 1 year old	
	Average IgG change (%)	P-value	Average IgA change (%)	P-value	Average IgM change (%)	P-value
Age one year	+18	<10 ⁻⁴	+27	<10 ⁻⁴	+58	0.08
Sex boys	baseline		baseline		baseline	
girls	+9	0.10	+3	0.74	+21	0.26
Breast feeding 3 months	+3	0.23	+12	0.002	+11	0.30
History of frequent infections negative	baseline		baseline		baseline	
positive	+4	0.31	+1	0.84	-12	0.51
DTP vaccine* one dose	+5	0.005	+4	0.09	+15	0.06
Sabin vaccine* one dose	+4	0.01	+3	0.22	+11	0.09

* alternatively introduced, to avoid collinearity problems.

lems (13, 14). The immunization schedule for children in Greece traditionally follows the recommendations of the American Academy of Pediatrics, namely 3 doses of DTP and Sabin vaccines during the first year of life, a first booster at 18 months and a second at the preschool age (15).

Results

Table 1 shows the frequency distribution of 414 subjects by the predictor variables.

Immunoglobulin G

Table 2 presents, for nine consecutive age groups, the respective number of subjects, the geometric mean and the log-normal range of IgG (as well as of IgA and IgM). The geometric mean is the antilog of the arithmetic mean of the log-transformed value and is, usually, very close to the median value. The normal range is derived by taking antilogs of the values determined by the formula: mean log values $\pm$ 2 standard deviations of the log values. Since there is random variation on account of biological variability, as well as laboratory variability, the age specific normal range should only be used as a crude yardstick. For IgG, visual inspection of the age-dependent changes in semi-log diagrammatic representation indicates that the age pattern of the log-transformed values can be reasonably described with two straight lines: one from 1 month to less than 5 years old and another from 5 to 14 years old.

A separate regression model was applied for each of these two age intervals with log-transformed val-

ues of IgG as dependent variables and a series of biologically plausible factors as independent (predictor) variables. The predictor factors were introduced continuously (age in years; and birth weight in 1000 gr), as ordered variables (DTP and Sabin vaccines, 0, 1, 2, 3, 4, 5 doses; and exclusively breast feeding, in intervals approximately equal to 3 months) or as categorical contrasts (sex; previous health status, fewer vs. more than 5 febrile episodes during the past year; and paternal occupation, manual vs. non-manual).

Tables 3 and 4 show the multiple regression derived proportional (percent) changes of IgG levels (as well as of IgA and IgM levels) among infants and children by indicated changes in a series of predictor variables. Since the values of the dependent variable were log-transformed the regression coefficients and the 95% confidence intervals represent proportional changes vs. the baseline value (in categorical contrasts) or per unit change (in quantitative or ordinal variables).

It can be seen (Table 3) that IgG levels increase by an average of 18% per year among children less than 5 year old and the increase is statistically highly significant. Girls appear to have 9% higher levels of IgG, although the difference is only marginally significant. There are also suggestions that do not reach statistical significance that birth weight (data not shown) and breast feeding are positively associated with IgG levels. Vaccination with DTP or Sabin is associated with 5% or 4% increase of IgG levels respectively, per dose, and past history of frequent infections with 4% increase, although the latter contrast is not statistically significant. In this study low

Table 4. Multiple regression-derived proportional (percent) changes of IgA and IgM levels among the respective older age group by indicated changes in a series of predictor variables

Age group Predictor Variable	5-14 years old		7-14 years old		1-14 years old	
	Average IgG change (%)	P-value	Average IgA change (%)	P-value	Average IgM change (%)	P-value
Age one year	+2	0.007	+4	0.01	+2	0.003
Sex boys	baseline		baseline		baseline	
girls	-4	0.41	-8	0.47	+15	0.02
Breast feeding 3 months	+2	0.21	+2	0.62	+3	0.25
History of frequent infections negative	baseline		baseline		baseline	
positive	+1	0.67	+3	0.66	-2	0.60
DTP vaccine* one dose	+0	0.77	+4	0.07	+1	0.73
Sabin vaccine* one dose	-0	0.76	+2	0.34	-1	0.58

* alternatively introduced, to avoid collinearity problems.

socioeconomic class, as reflected in manual paternal occupation, is not associated with IgG levels among children less than 5 years old (data not shown).

Concerning IgG levels among children more than 5 years old (Table 4), it can be seen that they increase by an average of 2% per year. The increase is statistically significant. No other association is statistically significant or strongly suggestive among older children, with respect to IgG.

Immunoglobulin A

Table 2 also shows, for nine consecutive age groups, the geometric mean and the log-normal range of IgA. Visual inspection of the age-dependent changes in semi-log diagrammatic representation indicates that the age pattern of the log-transformed IgA values can be reasonably described with two straight lines: one from 1 month to less than 7 years and another from 7 to 14 years.

Tables 3 and 4 show the multiple regression derived proportional (percent) changes of IgA levels among infants and children by indicated changes in a series of predictor variables.

In Table 3, it can be seen that IgA levels increase by an average of 27% per year among children 1 month to less than 7 years old and the increase is statistically highly significant. Sex and birth weight (data not shown) do not show statistically significant associations. Breast feeding is positively and statistically significantly associated with IgA levels. Vaccination with either DTP or Sabin is also positively but not statistically significantly associated with IgA levels. Past history of frequent infections and low

socioeconomic class (data not shown) are not significantly associated with IgA levels among children less than 7 years old.

Table 4 shows an increase of IgA levels by an average of 4% per year among children more than 7 years of age and the increase is statistically significant. No other of the studied variables is significantly associated with IgA levels in the age group 7 to 14 years.

Immunoglobulin M

Table 2 also shows, for nine consecutive age groups, the geometric mean and the log-normal range of IgM. For IgM, visual inspection of the age dependent changes in semi-log diagrammatic representation indicates that the age pattern of the log-transformed values can be reasonably described with two straight lines: one from 1 month to less than 1 year old and another from 1 to 14 years old.

Tables 3 and 4 show the multiple regression derived proportional (percent) changes of IgM levels among infants and children by indicated changes in the studied predictor variables.

In Table 3 there are statistically non significant but biologically meaningful suggestions that age and sex, as well as DTP and Sabin vaccinations, are positively associated with IgM levels in infants. History of frequent infections, breast feeding, birth weight (data not shown) and socioeconomic status (data not shown) were not significantly associated with IgM levels in infants in this study.

Table 4 shows an increase of IgM levels by an average of 2% per year among children more than 1

year old. The increase is statistically significant. Sex is also positively and statistically significantly associated with IgM levels. No other association with IgM levels is statistically significant or even suggestive among children 1 to 14 years old.

Discussion

The sample in the present study was not randomly chosen from any specified population, but selection factors related to the catchment area of the University Hospital are not likely to be related to immunoglobulin levels in a way that would compromise the validity of the results. Conditions that have necessitated the minor surgical operations for which the children were treated have not been associated with abnormal immunoglobulin values.

Several studies concerning the association of immunoglobulin levels with age have been reported in the international literature but there is no agreement with respect to age dependent patterns (3, 5–8, 10, 11). Part of the variability among studies may be due to different laboratory or statistical procedures. However, genuine variation may exist, reflecting different environmental conditions and in particular different exposures to infectious agents. There have been two previous studies in Greece (5, 6) both on smaller samples of children; they were undertaken about 20 years ago and immunodiffusion procedures were used to determine immunoglobulin levels. Neither of these studies has examined factors other than age and sex or has utilized modern, computer-dependent statistical techniques. It seems reasonable to assume that the results of the present study better describe the age dependent patterns of immunoglobulins G, A and M in the Greek paediatric population.

In agreement with several previous studies, IgM levels were higher in girls than in boys (1, 3, 5–7, 16), whereas there were no sex-related differences with respect to the two other immunoglobulin classes. Levels of all three immunoglobulin classes increased rapidly among younger children and slower thereafter, reaching asymptotically adult levels by the age of 14 years. However, the turning point was 5 years for IgG, 7 years for IgA and around 12 months of age for IgM.

Among infants and younger children, vaccination with either DTP or Sabin was associated with higher levels of all three immunoglobulin classes, although the results were statistically significant only with respect to IgG. The vaccination dependent differences were evident during the period of life characterized by rapid increase of the corresponding immunoglobulin: up to 5 years for IgG, 7 years for IgA and 12 months for IgM. As expected, vaccination accelerates the rate of increase of immunoglobulin

levels, but has no noticeable impact on these levels among older children. Substantial increases of vaccine specific antibodies have been demonstrated using highly sensitive laboratory procedures (17–19), but we have found no reports of total IgG increases in relation to vaccination status.

Breast feeding was positively associated with levels of IgA among young children, whereas there was no association among older children or with IgG and IgM immunoglobulins at any age. The differential pattern of association may be accounted for by the secretory nature of IgA antibodies, by the capacity of infants to absorb IgA from the milk of their mothers (20) and by stimulation through growth factors in breast milk (21). Similar results have been reported by some (20) but not all previous investigators (22, 23).

The results of this study illustrate the age and sex dependent patterns of immunoglobulins G, A and M in the contemporary Greek paediatric population and demonstrate the biologically predictable influences of vaccination and breast feeding on the levels of these immunoglobulins.

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