SERUM IMMUNOGLOBULIN LEVELS IN HEALTHY ETHIOPIAN SCHOOL CHILDREN

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Abstract

Serum concentrations of immunoglobulins IgG, IgA and IgM in 258 healthy Ethiopian school children and 82 healthy adults residing in Addis Abeba were measured by the radial immunodiffusion technique. The results are presented as geometric mean values in mg/100 ml and as percent of the adult mean values. A statistical analysis showed that the IgG and IgA concentrations at ages 8 and 7 respectively were lower than the adult values. The inference drawn from these results is that the IgG and IgA levels do not reach the adult level until after the age of 8 and 7 respectively. In contrast the IgM level was found to reach the adult level before the age of 7. No truly significant differences were observed in immunoglobulin levels which could be attributed to sex, intestinal parasitic infection or socio-economic status. The mean values for IgG and IgM obtained from this study were found to be higher than the reference values being used in the country at the moment. It is thus suggested that mean immunoglobulin concentration values obtained from this study be used as reference values until further study is made and more refined reference values are set.

1. INTRODUCTION

The measurement of immunoglobulins IgG, IgA and IgM in human sera is a common procedure in clinical laboratories and is useful both for clinical and research purposes. Data provided by measurement of immunoglobulins are important for the diagnosis of different kinds of disease. But due to the fact that the ranges of serum immunoglobulins vary in populations of different countries, valid conclusions cannot always be drawn from interpretations of results; unless the results are evaluated in the light of normal ranges of the populations under consideration. It is, therefore, important for different populations to have their own normal values for reference. The objective of this study is thus to establish normal values of serum immunoglobulins, namely IgG, IgA, and IgM, in Ethiopian school children residing in Addis Abeba.

Like all other normal values for physiological and biochemical parameters, the establishment of normal values for immunoglobulins in a given population is important from two points of view. First, such values are of clinical importance in the identification of abnormalities; and, secondly, if the normal ranges are found to be different from that reported in other countries this will lead to further studies to elucidate the causes for the differences.

The normal values quoted most of the time for immunoglobulin levels are the normal values of Caucasians living either in Europe

or North America. But there is a high incidence of raised immunoglobulin levels in Africans compared with Europeans or North Americans (17, 22, 38, 60, 77). Therefore Caucasian normal immunoglobulin values cannot be used for African populations. Even the existing reports on the immunoglobulins of Africans may not be useful for other African countries, for the very reason that conditions vary from one African region to another bringing about differences in the immunoglobulin levels. It is thus important to determine immunoglobulin levels in healthy Ethiopian school children residing in Addis Abeba and elsewhere in order to provide normal values which will be used as reference values in interpreting quantitative immunoglobulin estimations of patients.

Before going into the details of how this study was carried out, it may not be out of place to give some background knowledge of what immunoglobulins are and to further discuss why normal values for immunoglobulins have to be established.

1.1. Immunoglobulins: Structure, Properties and Functions

The word "immunoglobulins" (Igs) which was first proposed by Hermans in 1957 (34) has been given many different kinds of definitions. But the 1964 definition WHO (82) gave seems to be the most widely used. This definition of immunoglobulins follows:-

"Immunoglobulins are proteins of animal origin endowed with known antibody activity, and certain proteins related to them by chemical structure and hence antigenic specificity ..... Immunoglobulins are not restricted to the plasma but may be found in other body fluids of tissues, such as urine, spinal fluid, lymph nodes, spleen, etc. Immunoglobulins do not include the components of the complement system."

The structure of the basic immunoglobulin molecule consists of four polypeptide chains, two identical heavy chains and two identical light chains, joined to each other by a variable number of disulphide bonds (see Figure 1).

Five classes of immunoglobulins are commonly identified in normal human serum based on the heavy polypeptide chain differences (68). These classes are designated by the letters G, A, M, D and E preceded by the abbreviation Ig, indicating their immunoglobulin function or γ -indicating their electrophoretic mobility as gamma globulins (82).

A separate subdivision of human immunoglobulins is made on the basis of differences in the light polypeptide chains. Two forms of light chains are recognized and are identified as "Kappa" and "Lambda" chains. Immunoglobulins with "Kappa" chains are type K and molecules with "Lambda" chains are type L. The features that distinguish type-K from type-L are independent of the features that identify the long polypeptide chains. Only

one of the two classes of the light chains, K or L, is found in a given immunoglobulin molecule. That is to say some IgG molecules, for example, are type-K and others are type-L on the basis of the particular light polypeptide chain they have.

Each class of human immunoglobulin can be identified by specific antiserum raised against it. These antisera, which react with the specific antigenic determinants, also permit quantitative determination of each form of immunoglobulin.

Each immunoglobulin heavy polypeptide chain has distinct structure, properties and biologic functions. Some of these properties of immunoglobulins IgG, IgA and IgM are summarized in Table I.

The following comments are just supplementary information on the properties and functions of immunoglobulins IgG, IgA and IgM.

IgG:- is the only one of the five immunoglobulins known to cross the placenta, (13, 27, 80). The IgG molecules are actively transported across the placenta and provide the newborn with an abundant supply of maternal antibodies for the first few months of the neonatal period. IgG molecules seem to particularly arise in response to soluble antigens, such as bacterial toxins, and usually appear after an initial response of antibodies in the IgM class (39). The neutralization of the soluble products diffusing away from intruding cells seems to be largely the prerogative of IgG antibodies. In secondary immune responses,

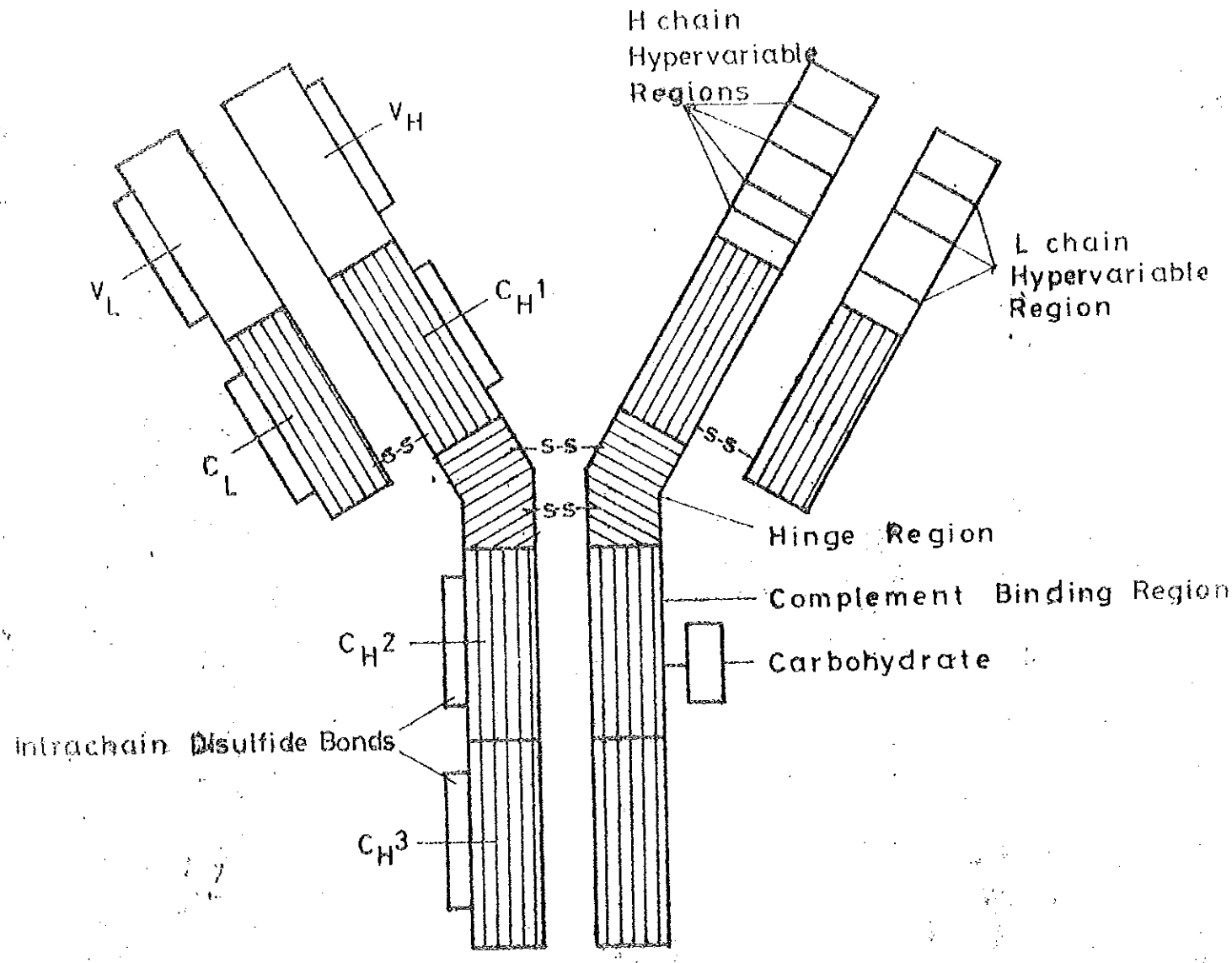

FIGURE 1 Schematic representation of IgG molecule showing the relatively variable [ ] and constant [▓] regions. V_L and C_L are variable and constant regions on the light chain. V_H and C_H specify variable and constant regions on the heavy chain. Both serum IgA and IgM have similar basic structure except that IgM has pentameric (5units) structure joined by a single J-chain.

like malaria and helminthic infection, very large quantities of IgG are produced (61).

IgA:- Unlike IgG, IgA does not cross the placenta; however it contributes to the immunity of the newborn by virtue of its high concentration in the colostrum. IgA's most important contribution to the immunity of an individual is in the external secretory system. It is vital in the defense of the gut against the enterovirus (10). The overall evidence collected by Hermans (36) shows that IgA plays a major role in acting as "antiseptic paint" of the mucous membrane. It may function by covering parts of the surface of pathogens and thus inhibiting their adherence to surface mucosal cells and hence their entry to the body.

IgM:- This class of immunoglobulins may be regarded as the most primitive of the immunoglobulins (61); because it is the first antibody that is produced in response to an antigen in the primary immune response, and because of the fact that in human gestation it is the first immunoglobulin to be produced in the fetus in response to infections. IgM is the first line of defense in the systematic circulation, large quantities of which are produced in response to particulate antigens like those of malaria, trypanosomiasis, helminths etc. (39). IgM in the circulation is confined to it and has a major role in the protection of the same. It may also offer the second humoral line of defense in the gut.

TABLE I

Properties of Human Immunoglobuline IgG, IgA and IgM

CLASS	IgG	IgA	IgM
Molecular Weight	160,000	170,000-500,000	900,000
Heavy Chain Designations			
Light Chains	K(K)+L(λ)	K(K)+L(λ)	K(K)+L(λ)
Sedimentation coefficient	7S	7S, 9S, 11S	19S
Number of Sub-classes	4	2	1
Antigen binding sites	2	2	5(10)
Half life (days)	18 - 23	5 - 6.5	5
Synthetic rate gm/day/70 kg	2.3	2.7	6.4
% of total immunoglobulin	80	13	6
Carbohydrate content	3	10	10
Serum concentration, mg/100 ml	600-1800	150 - 500	60 - 200
Placental Transfer	YES	NO	NO
Characteristic antibody	Precipitins antitoxins	Surface protection	Agglutinis Opsonins
Properties	Complement fixation Late antibody	Secretory antibody	Lysins Complement fixation early antibody

The information in this table was obtained from:

Belanti (9) Bowry (12) Dosgupta (25) Roitt (68) and Freedman & Gold (31).

1.2. Normal Values of Serum Immunoglobulin Levels and Factors Affecting them

As pointed out earlier in this paper, the establishment of local normal values of serum immunoglobulin levels is indispensible for the successful detection of abnormalities. But the reported normal serum levels have shown considerable differences from author to author. This may be due to variations of methodology, selection of subjects, and standards used or variations of analysis of data. The various immunological techniques used for the assay of immunoglobulins by different authors could give different results thus leading to differences in the normal values reported. Unless sera are obtained from subjects free of certain acute or chronic disease, for which immunoglobulins are known to be affected, the results obtained will not be normal serum levels. The commercial standard sera used by different workers could be different unless obtained from the same source. This will also contribute to the variations of the reported values. Therefore the main cause for the differences of the normal serum immunoglobulin levels reported by different workers, seems to be lack of uniformity. Other differences of normal values could arise due to real differences between populations. There are many factors that affect serum immunoglobulin levels and thus contribute to differences in normal values among populations. Some of these factors are as follows:-

A. Environment

The development of normal immunoglobulin levels is the result of host reaction to his environment. Observations on germfree animals indicate that these animals have very low levels of immunoglobulins (28), and, therefore, it is believed that the environmental challenge is largely what maintains even what is called the "Normal" level. Environmental factors like seasonal variation, altitude, climate, etc. have been reported for affecting immunoglobulin levels in different populations (2, 60).

B. Diseases

It is not surprising to note that it is typical for all the immunoglobulins to show elevations in different diseases, since most generalized infections provide multiple antigenic challenges through many routes (85).

Helminth and protozoal diseases like filariasis (18, 16), schistosomiasis (6, 8, 17, 49), ascariasis (44), ancylostomiasis (61), malaria (24, 62, 75, 76, 83) sleeping sickness (20, 48, 59), Chagas' disease (50, 58) and amebiasis (1) are said to affect the levels of one or more of the immunoglobulins.

Certain bacterial diseases like pulmonary tuberculosis (54), syphilis and yaws (51, 84) and leprosy (61) are among those often reported to increase the immunoglobulin levels.

Other diseases like viral infections, liver diseases, autoimmune diseases, malignancies like Waldenstron's macroglobulinemia etc. are known to upset the immunoglobulin levels in man.

C. Sex and Age

The effect of sex and age on immunoglobulin levels has been a point of controversy over a long time. Some authors (15, 66, 71) have reported that females above the age of seven have higher IgM levels than males; others (14) believe that such differences do not exist. There is also no agreement among different authors on the age at which immunoglobulins reach their highest level. Many (4, 14, 38) believe that immunoglobulin levels increase until the age of one year for IgM, 7 years for IgG and 16 years for IgA. Steihm and Funderbergh (74) reported that adult levels of the immunoglobulins are attained at the age of twelve. Again others like Buckley and Dorsey (15) stated that the immunoglobulin levels do not reach their maximum levels until the age of thirty.

The effect of race on the serum immunoglobulin levels is also debatable. Buckely and associates (14) reported that no differences related to race occur in the levels of immunoglobulins. However Shulman and Gilich (70) stated that for ages six and above the immunoglobulin levels in Blacks were higher than in Whites.

1.3. Methods and Applicability of Immunoglobulin level Estimation

The first attempt to quantitatively survey serum levels of immunoglobulins in disease was reported by Hermans (35). To date, various immunological techniques have been developed for the quantitative measurements of the three immunoglobulins IgG, IgA and IgM (16, 29, 30, 32, 33, 41, 55, 73, 81). However the most widely used and the most economical assay at present utilizes the Radial Immunodiffusion technique (RID) (5, 11, 23, 45). Of the number of RID methods that have been described (38, 61, 63) the method described by Mancini et al. (56) is the most accepted and widely used one.

Applications of the RID procedure for the assay of immunoglobulins provides data of clinical importance for many diseases. It is especially useful as a screening procedure in the detection of certain immunologic deficiency states, the dysgammaglobulinemias, multiple myeloma, Waldenstroms macroglobulinemia and many other diseases which are known to up-set the immunoglobulin levels. McFarlane (61) in his excellent review of immunoglobulins in tropical countries, gives about twenty five different kinds of diseases in which serial estimation of immunoglobulins can provide invaluable diagnostic and prognostic information.

As pointed out earlier, diagnostic information obtained from the measurement of immunoglobulins must always be interpreted in relation to standard normal values. However, it does not seem

justified to speak of normal human immunoglobulin levels that can be useful for all human populations. This is due to the fact that immunoglobulin levels are affected by changing and persistant environmental factors, disease, etc. bringing about wide ranges of differences among populations. Therefore for any given individual living under particular environmental conditions it is possible only to say whether or not his immunoglobulin levels fall within the average range of the population value of the region he lives in (65). This needs the establishment of normal values of immunoglobulin levels for a population in a region, and that is what this study is all about.

MATERIALS AND METHODS

Subjects

Serum samples were obtained from 340 (182 males and 158 females) apparently healthy subjects ranging from 7 - 47 years of age. 258 (114 males and 144 females) of these were students going to six schools in Addis Abeba; namely St. Josheph School, Nativity Boys' School, Nazereth School, Yehiwot Berhan School, Yekatit 23 and Medhanealem Elementary Schools. The rest of the subjects (68 males and 14 females) were adults; teachers and other personnel of the six schools, University students and staff.

Questionnaires were distributed to all the volunteer subjects in which they and/or their parents explained their health condition, from which the medical background of each subject was evaluated. Subjects with illness for which immunoglobulins were likely to be affected were excluded from the study. As well as this, the subjects were interviewed and the health of each individual was judged on the basis of freedom from complaints of acute or chronic disease and absence of obvious abnormalities. The monthly income of each subject and/or that of his parents was recorded. Subjects were grouped according to their incomes and those with monthly incomes of Birr 800 and above, and those with Birr 80 or less were chosen to be the groups to be compared to see the possible effect of socio-economic status on immunoglobulin

levels. These groups, the two extremes, were chosen for comparison in the hope that if at all any difference exists it will be clearly manifested.

Stool samples were collected from all the subjects in screw cap bottles with 5% formalin, and were examined for parasites using the Ritchie (67) concentration method.

Serum Sample Collection

2 - 5 ml of venous blood was collected (drawn) from one of the arms of each subject in an evacuated venoject tube by using a torniquet. The blood samples were allowed to stand at room temperature for about 4 hrs. to congulate. The tubes were centrifuged at 4000 r.p.m. for ten minutes and the sera were pipetted off and were immediately stored at - 20°C until analysis.

Immunoglobulin Concentration Measurement

Concentration of serum immunoglobulins IgG, IgA and IgM were determined by a modification of (40) the radial immunodiffusion (RID) method of Mancini et al. (56) utilizing commercially available rabbit antisera specific to human IgG (Y-chain specific), IgA (α-chain specific), and IgM (μ-chain specific) manufactured by Behring Institute (Behringwerke AG, Marbug, W. Germany). This method, briefly, consisted of precoating 8 cm glass plate with 0.5% agarose, onto which a

mixture of antisera and special noble agar (SIGMA) was to be layered. The proper amount of antisera as determined by A.H.R.I. (0.3 ml for IgG and IgM and 0.2 ml for IgA) was mixed with 12 ml of 2% noble agar in barbitone buffer. The antisera-agar mixture was then layered onto the pre-coated glass plate standing on a leveled surface and was allowed to set. After the agar had set 13 wells per plate were cut, using a template and gel punch which was attached to a water vacuum pump was used to remove the pieces of agar. Three of these wells were filled with 10 ml of the standard sera of known concentrations (dilutions) purchased with the antisera. The rest of the wells were filled with 10 µl each, of different serum samples, and the plates were left in humid chambers for 48 hrs (IgG and IgA) and 72 hrs (IgM) to equilibrate. Each plate was prepared in duplicate. The plates which formed precipitation rings after the appropriate time, were washed in Phosphate Buffer Saline (PBS) for 24 hrs, were then pressed, dried and stained with Commasie Brilliant Blue.

The diameters of the rings were measured with a measuring magnifier to the nearest 0.1 mm. Each duplicate sample was measured and the two results were averaged. A calibration curve was constructed for each plate by the least square technique to fit a linear regression line relationship between the log. concentration of the standard sera, whose concentrations

are already known, and the corresponding diameter of the ring. These calibrations were used to estimate concentrations of all the samples. The detailed procedure of the experimental work is found in Hudson and Hay's (40) Practical Immunology pp. 113 - 117.

For comparative purposes 32 samples of serum were randomly selected and the concentrations of immunoglobulins were determined by using both commercially available plates (Institute of Immunology and Virology, Torlak - Beograd) and plates made for this study. The results obtained in both cases were not significantly different from each other. This proved that the quality of the plates made by the methods employed in this work are as good as the commercial diffusion plates.

Statistical Analysis

Data obtained from this study were tested for logarithmic distribution by Davies (26) test. The result showed logarithmic distribution. Therefore all the values were log. transformed and means and standard deviations were calculated for the logarithmic forms. The logarithmic forms were converted back to the original arithmetic form for presentation. Students t-test (72) was used for the evaluation of significance.

RESULTS

IMMUNOGLOBULIN LEVELS IN NORMAL SUBJECTS

The data presented in Table II show the concentrations of immunoglobulins IgG, IgA and IgM in 340 apparently healthy subjects at different ages as geometric means. The arithmetic means plus or minus the standard deviations ($\pm$ S.D) of the individual groups, the % adult levels and the absolute ranges are listed in Table III for comparison with the geometric means.

Figure 2 depicts the geometric means and the normal limits of immunoglobulins IgG, IgA and IgM for different age groups. This figure shows the normal limits obtained by taking the means $\pm$ 2S.D. of the individual groups and then the antilogs of the results, which are much wider than the ranges given in Table II.

AGE EFFECTS ON IMMUNOGLOBULIN LEVELS

The results in Table II indicate that the IgG levels at 7 and 8 years of age are significantly lower than the adult level ($P < 0.001$ and $P < 0.05$ respectively). These findings show that the adult IgG level is not attained until after the age of 8 years. The IgA level at the age of 7 years is also significantly lower ($P < 0.05$) than the adult level; indicating that it reaches the adult level at this age and atabilizes thereafter. The IgM level at 7 years of age is not different from the adult level showing that it reaches the adult level at least before the age of seven.

SEX RELATED DIFFERENCES

Table IV shows the serum immunoglobulin levels in males and females at different ages. There was no statistically significant difference ($P \geq 0.1$) in immunoglobulin levels that can be attributed to sex. However, as shown in Figure 3, the IgM levels at all ages were found to be higher in females than in males. But these differences were not statistically significant.

EFFECTS OF INTESTINAL PARASITES AND SOCIO-ECONOMIC STATUS

The immunoglobulins IgG, IgA and Igm levels in individuals with intestinal parasites are compared to Normal subjects as shown in Table V. No statistically significant differences were observed between the two groups. As shown in Table VI immunoglobulin levels in two groups of subjects with different monthly incomes (socio-economic status) were compared and statistical analyses showed hardly any difference between the levels of immunoglobulins of the groups.

COMPARISON OF THE RESULTS OF THE PRESENT STUDY WITH OTHER VALUES

Table VII shows the serum immunoglobulin levels in Ethiopians compared with levels reported in other populations. Conclusive statements cannot be drawn from what is shown in the table. The table is presented only to show the differences and similarities of the values between populations. In Table VIII the normal serum immunoglobulin levels reference values being used in

Ethiopia at the moment are compared with values obtained from this study. The ranges of IgG and IgM reference values were found to be lower than the results of this study, suggesting that these are not the appropriate reference values for the Ethiopian population. Mean values and two different kinds of ranges of serum immunoglobulin levels in healthy Ethiopian subjects at different ages are listed in Table IX. These values may serve as reference values for Ethiopians.

TABLE II

Levels of Serum Immunoglobulins in 340 Normal Subjects at different ages

Age	Number of Subjects	Level of IgG		Level of IgA		Level of IgM	
		a Mg/100 ml	% of Adult Level	a Mg/100 ml	% of Adult Level	a Mg/100 ml	% of Adult Level
7	16	882 (527 - 1476)	54	176 (103 - 302)	76	159 (98 - 258)	95
8	30	1228 (647 - 2333)	76	194 (113 - 334)	85	143 (81 - 252)	86
9	31	1721 (819 - 3613)	106	211 (105 - 427)	91	194 (128 - 293)	116
10	37	1498 (839 - 2674)	92	219 (129 - 372)	94	164 (98 - 275)	98
11	37	1696 (815 - 3531)	105	200 (118 - 338)	86	161 (100 - 259)	96
12	18	2228 (1221- 4067)	136	193 (123 - 304)	83	161 (99 - 261)	96
13	32	1684 (1008 - 3382)	104	226 (123 - 408)	97	172 (106 - 278)	103
14	20	1460 (781 - 2731)	88	252 (153 - 408)	109	179 (116 - 271)	107

TABLE II
(Cont'd)

Age	Number of Subjects	Level of IgG		Level of IgA		Level of IgM	
		Mg/100 ml [a]	% of Adult Level	Mg/100 ml [a]	% of Adult Level	Mg/100 ml [a]	% of Adult Level
15	12	1737 (963 - 3130)	107	220 (129 - 375)	95	183 (103 - 325)	110
16	25	1610 (1865 - 2998)	99	225 (135 - 375)	97	176 (121 - 280)	105
Adult (17 & above)	82	1620 (885 - 2967)	100	232 (141 - 386)	100	167 (99 - 280)	100

[a] Immunoglobulin levels are expressed as geometric means. The ranges given in parenthesis, are obtained by taking the mean logarithm ± 1 standard deviation of the individual group and then taking the antilogs of the results.

TABLE III

Levels of Serum Immunoglobulins in 340 Normal Subjects at different ages

Age	Number of Subjects	Level of IgG		Level of IgA		Level of IgM	
		a Mg/100 ml	% of Adult Level	a Mg/100 ml	% of Adult Level	a Mg/100 ml	% of Adult Level
7	16	1073 ± 380 (515 - 1567)	51	172 ± 99 (84 - 285)	66	177 ± 90 (77 - 377)	94
8	30	1513 ± 1109 (515 - 5297)	72	223 ± 124 (68 - 631)	85	166 ± 106 (51 - 366)	88
9	31	2170 ± 1284 (366 - 5297)	103	247 ± 184 (89 - 624)	95	210 ± 81 (50 - 390)	112
10	37	1755 ± 1027 (433 - 4295)	84	251 ± 167 (68 - 417)	96	189 ± 118 (62 - 608)	101
11	37	2178 ± 1182 (313 - 9289)	104	226 ± 109 (48 - 444)	87	180 ± 98 (74 - 562)	96
12	18	2586 ± 1387 (585 - 5675)	123	212 ± 98 (44 - 440)	81	179 ± 93 (62 - 450)	95
13	32	2186 ± 1317 (418 - 6235)	104	267 ± 165 (98 ± 668)	102	192 ± 101 (94 - 425)	102
14	20	1733 ± 1076 (478 - 4805)	83	276 ± 136 (96 - 551)	106	195 ± 95 (96 - 387)	104

TABLE III

(Cont'd)

Age	Number of Subjects	Level of IgG		Level of IgA		Level of IgM	
		Mg/100 ml [a]	% of Adult Level	Mg/100 ml [a]	% of Adult Level	Mg/100 ml [a]	% of Adult Level
15	12	2010 ± 1276 (776 - 4805)	96	245 ± 117 (67 - 505)	94	212 ± 124 (87 - 502)	113
16	25	1895 ± 853 (611 - 4395)	90	251 ± 125 (67 - 574)	96	199 ± 84 (83 - 399)	106
Adult (17 & above)	82	2100 ± 1310 (586 - 5333)	100	261 ± 146) (100 - 640)	100	188 ± 103 (61 - 535)	100

[a] Arithmetic means ± S.D. are presented here. The Standard deviations (S.D) are the actual Standard deviations of the individual groups. The ranges, given in parenthesis are the actual observed ranges. Comparison of this table with Table - I will show how much difference the Geometric and Arithematic Means have.

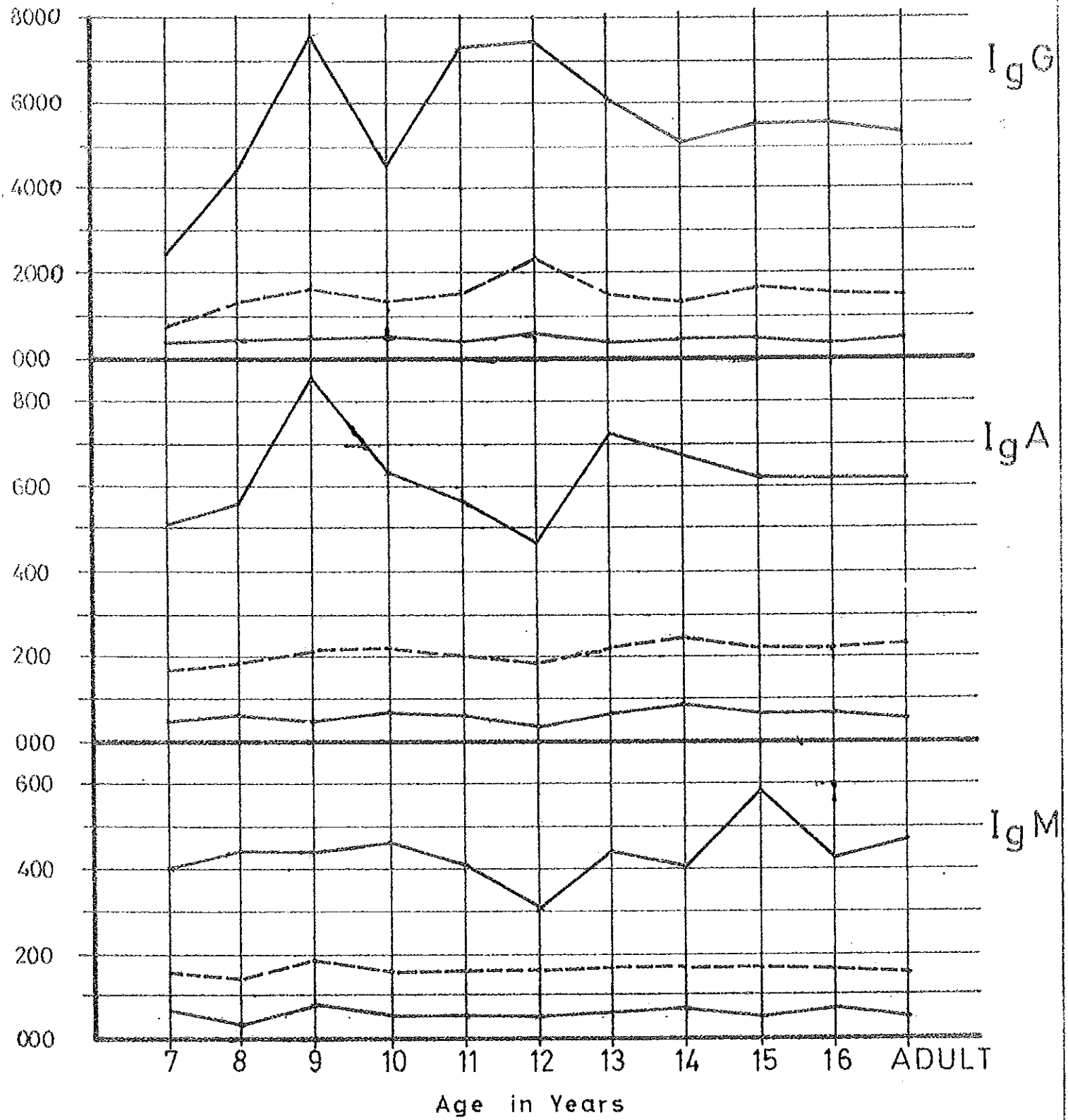

FIGURE 2 The broken lines(----) show the mean values obtained from table II. The bold lines show upper and lower boundries obtained by taking the mean log ± 2S.D. of the individual groups and then taking the antilogs of the results. This figure shows how wide the ranges of the immunoglobulin levels are.

TABLE IV

Serum Immunoglobulin Levels in Males and Females at different ages

Age	Number of Subjects		Level of IgG mg/100 ml[a]			Level of IgA in Mg/100 ml[a]			Level of IgM in Mg/100 ml[a]		
	Male	Female	Male	Female	Significance of difference between sexes	Male	Female	Significance of difference between sexes	Male	Female	Significance of differenc between sexe
7	6	10	1111	773	N.S.	137	205	N.S.	145	170	N.S.
			(828 -1490)	(437 -1364)	(P > 0.1)	(70 -272)	(59 -704)	(P > 0.1)	(95 -226)	(99 -290)	(P > 0.5)
8	14	16	822	1746		201	189	N.S.	103	192	
			(606 -1114)	(909 -3353)	(P < 0.001)	(11 -364)	(116-307)	(P > 0.1)	(68 -154)	(114-322)	(P < 0.005)
9	8	23	1137	1983	(P > 0.05)	196	217	N.S.	136	220	
			(814 -1587)	(903 -4375)	(P > 0.05)	(150-255)	(97 -483)	(P > 0.1)	(91-205)	(157-308)	(P < 0.005)
10	15	22	2019	1867		206	227	N.S.	144	180	N.S.
			(662 -1775)	(1100-3168)	(P < 0.005)	(128-339)	(130-395)	(P > 0.1)	(82-253)	(113-288)	(P > 0.200)
11	18	19	1453	1802	N.S.	206	191	N.S.	143	180	N.S.
			(1030-2048)	(736-4413)	(P > 0.1)	(118-360)	(117-311)	(P > 0.1)	(92-222)	(111-291)	(P > 0.1)
12	8	10	1673	2802	N.S.	175	209	N.S.	154	167	N.S.
			(913-3066)	(1716-4574)	(P > 0.05)	(121-252)	(128-344)	(P > 0.1)	(91-259)	(106-261)	(P > 0.5)
13	17	15	1644	2105	N.S.	205	254	N.S.	162	184	N.S.
			(831-3253)	(1314-3370)	(P > 0.2)	(117-358)	(139-467)	(P > 0.1)	(103-254)	(116-293)	(P > 0.4)

TABLE IV

(Cont'd)

Age	Number of Subjects		Level of IgG mg/100 ml[a]			Level of IgA in mg/100 ml[a]			Level of IgM in Mg/100 ml[a]		
	Male	Female	Male	Female	Significance of difference between sexes	Male	Female	Significance of difference between sexes	Male	Female	of difference between sexes
14	11	9	1283 (604-2723)	1711 (1188-2463)	N.S. (P > 0.2)	243 (140-423)	263 (172-402)	N.S. (P > 0.1)	158 (102-244)	210 (145-305)	N.S. (P > 0.1)
15	8	4	1789 (855-3742)	1711 (1040-2814)	N.S. (P > 0.5)	155 (86 -282)	269 (185-391)	N.S. (P > 0.1)	126 (97 -165)	220 (126-385)	(P > 0.05)
16	9	16	1833 (961-3499)	1497 (826-2714)	N.S. (P > 0.5)	206 (146-291)	236 (133-422)	N.S. (P > 0.1)	152 (99 -233)	205 (141-298)	N.S. (P > 0.05)
	68	14	1176 (1092-2888)	1754 (876-3513)	N.S. (P > 0.5)	231 (142-376)	236 (135-410)	N.S. (P > 0.1)	162 (97 -270)	196 (128-300)	N.S. (P > 0.2)

[a] Means and ranges were obtained as in TABLE II

N.S. = Not Significant

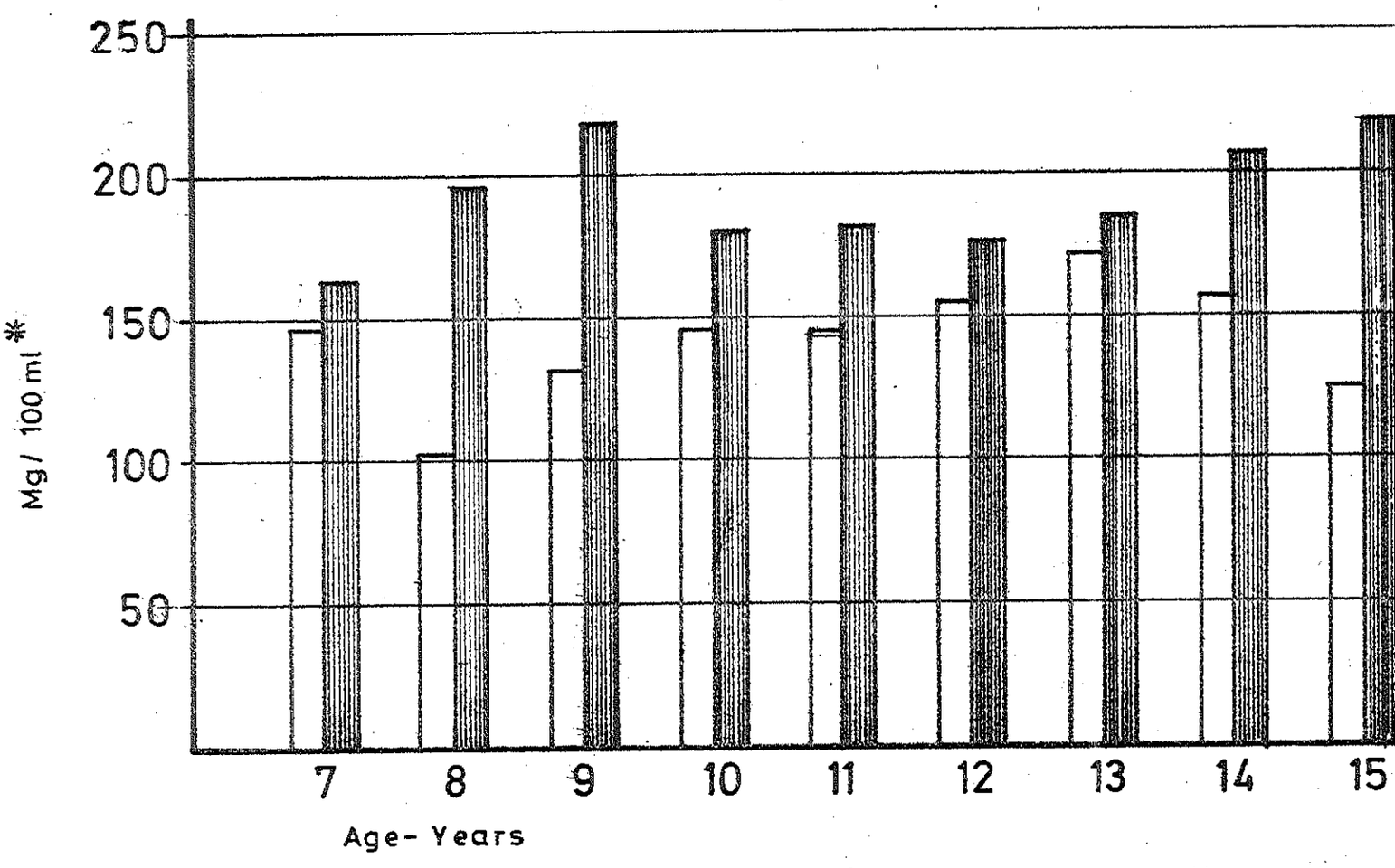

FIGURE 3 Graph showing the mean levels of igm in males and fem
The females are showen to have higher level of IgM than the male

TABLE V

Analysis of Immunoglobulin Levels in Individuals with Intestinal Parasit

Type of Parasite	No. of Parasitized individuals	IgG Level Mg/100 ml[a]	Significance of diff. from Normal Subjects	IgA Level Mg/100 ml[a]	Significance of diff. from Normal Subjects	IgM Mg/1
Ascaris	52	1665 (885-3130)	N.S. (P > 0.2)	240 (154-372)	N.S. (P > 0.1)	1 (104
Trichuris	10	1334 (784-2267)	N.S. (P > 0.5	235 (157-354)	N.S. (P > 0.5)	1 (105
Ascaris & Trichuris	14	1369 (558-3186)	N.S. (P > 0.5)	217 (138-341)	N.S. (P > 0.5)	1 (114
Strongyloides	4	1419 (656-3074)	N.S. (P > 0.5)	304 (249-372)	N.S. (P > 0.1)	2 (153
Giardia	9	1199 (627-2279)	N.S. (P > 0.5)	239 (159-360)	N.S. (P > 0.5)	1 (99
Normal Subjects	50	1374 (423-4464)		214 (130-353)		1 (147

[a] Means and ranges are obtained as in Table II.

TABLE VI

Analysis of Serum Immunoglobulin Levels in Subjects* with Different Socio-economic

Family Income per month	No. of Subjects	IgG mg/100 ml**	IgA mg/100 ml**	IgM
Birr 80 or less	57	1801 (957 - 3392)	246 (143 - 421)	(1
Birr 800 and above	57	1850 (1045 - 3274)	249 (156 - 382)	(1
Significance of difference		N.S. P ≥ 0.5	N.S. P ≥ 0.5	

*All Subjects are of ages 9 and above

**Mean values are geometric means and the ranges, in parenthesis, are obtained as in TABLE II

TABLE VII

Serum Immunoglobulin Levels in Ethiopians Compared with values in other po

Reference	Country	Race	Number of Subjects	IgG Mg/100 ml	IgA Mg/
A. Segovia & Fishbein (2)	Acapulco (Mexico)	Mixed	38	1456 (700 -2520)	20 (108 -
Michaux (64)	Congo	Black	112	2151 (1135-5220)	18 (70 -
A. Segovia & Fishbein (2)	Mexico, Mexico	Mixed	38	1456 (700 -2520)	20 (108 -
A. Segovia & Fishbein (2)	Mexico, Mexico city	Mixed	38	1549 (870 -2560)	21 (68 -
Wells (79)	New Guinea	Non-Watut Aborigine	30	1919 (1370-2640)	14 (86 -
		Watut Aborigine	12	3657 (3097-3937)	23 (157 -
McFarlane (60)	Nigerian	Black	65	1647 (1197-2222)	28 (135 -
Avends and Gallango (7)	Venezuela	Mixed	36		

TABLE VII

(Cont'd)

Reference	Country	Race	Number of Subjects	IgG Mg/100 ml	IgA Mg/1
Buckley et al. (14)	U.S.A.	White	23	982	245
		Black	7	1372	349
Allansmith et al. (4)	U.S.A.	Mixed	315	1045	170
				(710 - 1540)	(60 - 4
The Present Study	Ethiopia[a]	Black	326	1700	217
				(781 - 3613)	(105 - 4

[a] All the values are adult values, but since no significant difference is found between the adult and children above the age of 8 in this st the means and ranges age combined for the Ethiopian Subjects.

TABLE VIII

Comparision of Normal Serum Immunoglobulin Level Reference Values Being used in the Country at the Moment with Values Obtained from this Study

	IgG Mg/100 ml	IgA Mg/100 ml	IgM Mg/100
Currently used Reference Values[a]	800 - 1800	90 - 450	60 - 250 70 - 280
Values obtained from this study[b]	781 - 3613 (1700)	105 - 427 (217)	81 - 325 (169)

[a] Copied from Central Laboratory and Research Institute (C.L.R.I.), Immunol Section Clinical findings Report Sheet.

[b] The values in parenthesis are geometric mean values, of ages 9 and above for IgG, ages 8 and above for IgA, and ages 7 and above for IgM. The range are obtained as in TABLE II.

TABLE IX

Suggested Reference values for Serum Immunoglobulin Levels in Healthy Ethiopian Subjects at different ages

AGE	IgG Mg/100 ml[a]	IgA Mg/100 ml[a]	IgM Mg/100 ml[a]
9 years and above	1700[a] (781 - 3613) (390 - 7588)	217 (105 - 427) (52 - 862)	169 (81 - 325) (46 - 578)
8 years	1228 (647 - 2333) (340 - 4433)	217 (105 - 427) (52 - 862)	169 (81 - 325) (46 - 578)
7 years	882 (527 - 1476) (315 - 2471)	176 (103 - 302) (66 - 572)	169 (81 - 325) (46 - 578)

[a] mean values are expressed as

DISCUSSION

The data obtained from the measurement of concentrations of IgG, IgA and IgM show a rather broad range. Such results are expected. In fact the range of values for serum immunoglobulins in "normal" subjects is so wide that some times the diagnostic value has even been questioned (70). However when these values are presented in geometric means the ranges become narrower than the absolute ranges. For instance, the range of IgG level at age 11 in Table III is 313 - 9289 mg/100 ml. The highest value here is about 30 times the lowest one. The same value presented as geometric mean $\pm$ 2S.D would show a narrower range of 410 - 7062 mg/100 ml, and the highest value is only about 18 times the lowest one. Therefore it is evident that the geometric mean $\pm$ 2 S.D. range is narrower than the absolute range. This happens because the geometric mean is not very much affected by extreme values. For reasons that will be explained later on it is more appropriate to use the geometric mean than the arithmetic mean in studies like this here and log transforming the values for statistical analysis is indispensable. Failure to consider these facts will lead to erroneous interpretations of both research data involving immunoglobulin levels and/or similar

Table III shows arithmetic means and the absolute ranges. Presenting both data is advantageous. For one thing the geometric mean is not as widely known as the arithmetic mean, and thus presenting the arithmetic mean can give a better idea to one who is not familiar with the geometric mean. The other thing is that the absolute ranges give an idea of how low or high immunoglobulin values can be in normal populations. Understanding the lower and upper limits of immunoglobulin levels in a population is particularly important while considering immunodeficiency cases like hypo - and hyper-gammaglobulinemia. As shown in Figure 2 the lower limits of immunoglobulin levels are less variable than the upper bounds. The possible explanation for this is the fact that there are more factors which contribute to increased immunoglobulin levels, like environmental factors, disease, etc., than factors which result in decreasing them. It is clear that one needs to consider both the means and the ranges of immunoglobulin levels while considering clinical cases. Although the mean $\pm$ S.D. range, which contains only 68% of the population value, is more commonly used than the mean $\pm$ 2 S.D. which is said to include 95% of the population value (72), since the values obtained from this study are intended

levels at 7 and 8 years of age are only 54 and 76% of the adult level respectively.

These are significantly lower than the adult levels. This indicates that the IgG level does not reach the adult level until after the age of eight. The IgA level at 7 years of age is 76% of the adult level which is again significantly lower than the adult level. This also shows that the IgA level does not stabilize until after the age of seven. These two findings are almost parallel to the results of the study of Buckley et al. (14) who reported that IgG reaches adult level at the age of 6 and adult IgA level is reached and preserved by age 7 and beyond. Others have variously stated that IgG reaches the adult level by the 1^{st} to 2^{nd} year (4), the second year (21), the third year (81), the fifth year (43), 15^{th} to 16^{th} year (57, 74) etc. IgA is also reported to reach adult level by 9 months (37), 5 years (42, 81) 4 - 12 years (4) of age.

At this point it is worth considering why there are such different reports. As pointed out earlier in this paper, differences could arise due to experimental methods employed, type of standards used etc. But even if similar experimental procedures are used, differences may still appear to exist due to

obtain absolute normal values, but also to analyze the data so that meaningful statistical comparisons can be made.

Different authors (3, 4, 16, 46) have stated that immunoglobulins do not conform to the normal Gaussian distribution. The data obtained from this study also showed logarithmic distribution when tested by Davies (26) test. It is for this reason that the values obtained in this study were log transformed for statistical analysis and that geometric means were used (47). The log transformed values followed a Gaussian distribution. In only a few previous studies (3, 14) of immunoglobulins were the data converted to logarithms. Unless data are approximately normally distributed and variances are found to be approximately equal in different groups, commonly used statistical techniques like the t-test are invalid (47, 72). Lack of appropriate statistical analysis, such as the one mentioned above, is one source of the mistake made in the information given, particularly with reference to conclusion about ages at which adult levels for immunoglobulins are reached (14).

The level of IgM of the children at the age of seven is not different from the adult level, and this suggests that the adult level is attained at the age of seven or earlier. IgM

immunoblobulins. Further study has to be done to detect the age at which IgM level reaches the adult stage. However the results of this study show that age distinction need not be made after the age of 9, 8, and 7 for IgG, IgA and IgM respectively, when interpreting results.

Although the IgG level at 8 years of age and the IgM levels at 8 and 9 years of age are significantly higher in females than in males; and the IgG level in males is significantly higher than in females at the age of 10, the overall results of this study show that sex does not have a significant effect on immunoglobulins. The IgM level in females is seen to be consistently higher than in males, however this difference is not statistically significant. Some authors (4, 66) reported that the IgM level is higher in females than in males. Buckley and associates (14), Johanson and collaborators (44), and Cejka and co-workers (19) suggested that there was no significant difference between sexes. It therefore appears that immunoglobulin levels are possibly not significantly affected by sex and the values can be used without sex distinction. But the reasons for the differences of IgG levels at ages 8 and 10 and IgM levels at ages 8 and 9 between sexes obtained here has to be explained. Since these results

the IgM level differences between the sexes are not significant, why such differences occur and whether or not these differences affect the immune mechanism of the subjects remains to be an open door for further investigation;

Intestinal parasties are common inhabitants of the gut in normal children in the tropics. These parasites may bring about certain physiological alterations in the body of the hosts. Immunoglobulin levels may also be affected by parasites. Johansson and associates (44) reported that immunoglobulin E level differences between Ethiopian and Swedish children were due to Ascariasis in the Ethiopians. The results of this study do not show significant difference in immunoglobulin levels between subjects with Ascaris and the normal subjects. McFarlane (61) also reported that elevated serum immunoglobulin levels were observed in subjects with hookworm anemia. None of the subjects of this study was positive for hookworm. This is obvious because of the fact that ancylostomiasis is an occupational infection commonly found in moist agricultural soils. One would not expect that ancylostomiasis could be one of the few parasites that were found in the subjects of this study. Therefore no comment can be made in relation to McFarlane's (61) state-

from this result is that the parasitic infections shown in Table V do not affect IgG, IgA or IgM levels.

Socio-economic status differences among subjects of this study did not show significant differences in the immunoglobulin levels. This finding agrees with the reports of Lichtman and co-workers (52, 53) which asserted that gammaglobulin levels were not affected by social class (socio-economic status). However the possibility remains that this factor may play a role in affecting the immunoglobulin levels in association with other socio-cultural factors.

As the aim of this study was to demonstrate immunoglobulin levels, the question of the effect of parasitic infection, socio-economic status, etc. needs further study. However, the immunoglobulin levels in healthy Ethiopian school children were not affected by sex, intestinal parasites, or socio-economic status. Therefore it is possible to suggest a working framework of reference values of immunoglobulins which can be used in Ethiopia and other areas with similar environmental conditions without making any distinction of sex, socio-economic status, or parasitic infection.

The serum immunoglobulin level values for Ethiopians compared with values from other populations clearly indicate that

regions. Thus Ethiopians living in the lowland region where malaria is found may have higher IgG values, probably nearer to the Nigerians or Congolese than to the values shown here. Direct comparisons and conclusive statements cannot be made from these values of other populations for the experimental methods, choice of subjects, statistical analysis, etc. could be very different from the ones used in this study. However the comparison shows that similarities and differences exist among values of different populations and thus the reference value of one region cannot be used for another one without discrepancies. Therefore the idea of establishing normal values for every population is not questionable.

Finally it is important to comment on the normal reference values used in Ethiopia at the moment, in the light of the findings of this study. The reference values being used in this country at the moment for clinical services are quite different from the results of the present study. The IgG and IgM values of the reference are much lower than the values obtained from this study.

No wonder, the so called reference values must have been copied from a textbook or commercial RID plates' instruction leaflets, which show levels of Caucasians in America or Europe.

Therefore, it is appropriate to drop these reference values

differences in immunoglobulin levels exist between residents of Addis Abeba and others living under different environmental conditions, and in the meantime the suggested normal reference value for IgG, IgA and IgM concentrations in Table IX can be used for the Ethiopian population in Addis Abeba or similar towns of the same environment.

REFERENCES

1. Abioye, A.A., Lewis, E.A. and McFarlane, M. Clinical evaluation of immunoglobulins in amoebiasis, Immunology, 23, 937, 1972.

2. Alarcon-Segovia, D. and Fishbein, E. Demography of Serum Immunoglobulins: Differences in IgG and IgM levels in two normal Mexican adult populations, Clin. Sci. 39, 467, 1970.

3. Allansmith, M. Development of Immunity pp. 582-601, in: Falkner, G. (ed.) Human Development, W.B. Saunders, Philadelphia, 1966.

4. Allansmith, M., McClellan, B.H., Butterworth, M. and Maloney, J.R. The development of immunoglobulin levels in man. J. Pediat. 72, 276, 1968.

5. Arvan, D.A. Antigen-antibody crossed electrophoresis. Lab. Management 7, 21, 1969.

6. Atunes, L.J., Reis, A.P., Pellegrino, J., Tavares, C.A., and Kate, N., Immunoglobulins in human S. Mansoni. J. Parasitol. 57, 539, 1971.

7. Avends, T., and Gallango, M.L. Immunoglobulin levels in blood

8. Bassily, S., Higashi, G.I., Farid, Z., and Williams, R.E. Serum Immunoglobulins and Schistosoma Mansoni. J. Trop. Med. Myg. 75, 73, 1972.

9. Belanti, J.A. Immunology II, 2nd edn. W.B. Saunders Company Philadelphia, 1978.

10. Berger, H., Ainbender, E., Hodes, H.C., Zepp, H.D., and Hevizy, M.M. Demonstration of Serum IgA polio-antibody in saliva, duodenal fluid and urine. Nature, 214, 420, 1967.

11. Berne, B.H. Differing methodology and equations used in quantitating immunoglobulins by radial immunodiffusion. A comparative evaluation of reported and commercial techniques. Clin. Chem. 20, 61, 1974.

12. Bowry, T.B Immunology Simplified Oxford University Press, Oxford, 1977.

13. Branbell, F.W. Passive immunity in Young mammals. Biol. Rev. 33, 488, 1958.

14. Buckley, R.H., Dees, S.C, and O. Fallon, W.M. Serum immunoglobulin levels in normal children and in uncomplicated allergy. Pediatrios, 41, 600, 1968.

16. Calman, H.N. and Merrill, D. Quantitative measurement of human gamma - 2, beta - 2A, and beta - 2M serum immunoglobulins. J. Lab. Clin. Med. 64, 685, 1964.

17. Cappuccinelli, P., Frentzel-Beyma, R.R., Martinello, P. and Sena, L. Serum proteins and immunoglobulins in adult Liberians. J. Trop. Med. Hyg. 75, 180, 1972.

18. Carlisle, R., Ogunba, E.D., McFarlane, H., Onayemi, O.K. and Oyeleye, V.O. Immunoglobulins and antibody to loa loa in Nigerians with endomyocardial fibrosis and other heart diseases. Brit. Heart J. 34, 678, 1972.

19. Cejaka, J., Mood, D.W. and Kim, C.S. Immunoglobulin concentrations in sera of Normal Children: Quantification against an international reference preparation. Clin Chem. 20, 656, 1974.

20. Charmot, G., Vargues, R., Bergot, J., Carrie, J.Y., Deu, J., Fouchet, M., Gueri, J.L., Reynoud, R., Rigand, I.C., and Soubre-Piere, P. L'etiologie des macroglobulin e mies observees en Afrique. Sem. Hep. 30, 1421, 1963.

21. Collins-Williams, C., Tkachyt. S.J., Toft, B. and Moscarello, M. Quantitative immunoglobulin levels in Children. Int.

23. Crowle, A.J. Immunodiffusion, 2nd edn. Academic, New York, 1973.

24. Curtain, C.C., Kidson, C., Champness, D.L., and Gormam, J.G. Malarial antibody content of gamma 75 globulin in tropical population, Nature 203, 1366, 1964.

25. Dasgupta, A. Modern Immunoglogy, Tata McGraw Mill, New Delhi.

26. Davies, G. Davies test for logarithmic distribution. pp 78 - 79. In: Langley, R. Practical Statistics, 2nd edn. Pan Books Ltd. London, 1970.

27. Fahey, J.L. Antibodies and Immunoglobulins, I. JAMA. 194, 71, 1965.

28. Fahey, J.L. Antibodies and Immunoglobulins. II Normal development and changes in disease. JAMA. 194, 255, 1965.

29. Fahey, J.L. and Lawrence, M.E. Quantitative determination of 6, S gamma-globulins, beta-2A globulins and gamma-1 macroglobulins in human serum. J. Immun. 91, 597, 1963.

30. Fahey, J.L. and McKelvey, E.M. Quantitative determination of serum immunoglobulins in antibody - agar plates. J. Immun. 94, 84, 1965.

32. Gell, P.G.H. The estimation of the individual human serum protein by an Immunological Method. J. Clin. Path 10, 67, 1957.

33. Grant, G.H., and Everall, P.H. Gel-immunofiltration, a new technique for the qualitative analysis of serum proteins. J. Clin. Pathol. 18, 654, 1965.

34. Hermans, J.F. Immunochemical studies on protein pathology. The immunoglobulin concept. Clin. Chem. Acta. 4, 639, 19 1957.

35. Hermans, J.F. "Les Globulines Seriques du Systeme Gamma", Arscia, Brussels, 1960.

36. Hermans, J.F. Immunoglobulin formation and function in different tissues. Curr. Trop. Microbiol. Immunol, 45, 131, 1968.

37. Hitzig, W.H. Die Plasmoproteine in der Klinischen Medizine. Springer, Berlin, 1968.

38. Hobbs, J.R. Immune globulins in some diseases. Brit. J. Med. 3, 669, 1970.

39. Hobbs, J.R. Immunoglobulins in Clinical Chemistry. Advances

41. Huntley, C.C. Simple gel. diffusion micromethod for gamma globulin determination, Pediatrics. 31, 123, 1963.

42. Huntley, C.C. and Lyerly, A. Immune globulin determinations in allergic children. Amer. J.Dis. Child. 106, 545, 1963.

43. Johansson, S.G.O. and Berg, T. Immunoglobulin levels in healthy children Acta Paediatrica Scandinavica. 56, 572, 1967.

44. Johansson, S.G.O., Melbin, T. and Vahlquist, B.O. Immunoglobulin levels in Ethiopia pre-school children with special reference to high concentration of immunoglobulin E. (IGND). Lancet, i, 1118, 1968.

45. Kalff, M.W. Quantitative determination of serum immunoglobulins by single radial immunodiffusion. Clin. Biochem. 3, 91, 1970.

46. Kohler, P.F. and Farr, R.S. Quantitative comparison of immunoglobulins in atopic (reagnic) and nonatopic (non-reagnic). Individuals: Higher gamma-D levels in atopic sera J. Alleng. 39, 311, 1967.

47. Langley, R. Practical Statistics, 2nd edn. Pan Book Ltd. London, 1970.

49. Lehman, J.S., Higoshi, G., Bassily, S. and Farid, Z. Rheumatoid factors in Salmonella and Schistosoma infections. Trans. Ray. Soc. Trop. Med. Hyg. 66, 125, 1972.

50. Lelchuck, R., Delmasso, A.O.P., Inglesina, C.L., Alvarev, M., and Cerisola, J.A., Immunoglobulin studies in serum of Patients with American trypanosomiasis (Chagas disease) Clin. Exp. Immunol. 6, 547, 1970.

51. Lesinska, M.W. and Jakubowski, A. Relationship of antibodies detected by the immunofluorescence method with the various classes of immunoglobulins in case of untreated syphilis. Brit. J. Vner. Dis. 46, 380, 1970.

52. Lichtman, M.A., Hames, C.G., and McDonough, J.R. Serum protein electrophoretic fractions among Negro and White subjects in Evans County, Georgia. Amer. J.Clin. Nutr. 16, 492, 1965.

53. Lichtman, M.A., Vaughan, J.H. and Hames, C.G. The distribution of Serum immunoglobulins, anti-gamma-G-Globulins ("rheumatoid factors") and antinuclear antibodies in white and Negro subjects in Evans County, Georgia.

55. Mancini, G., Vaerman, J.P., Carbonara, A.O., and Mermans, J.F. A single radial diffusion method for the immunological quantification of proteins. Proceedings of the 11th colloquium, porotides of the Biological fluids, Amsterdam, Elsevier, 370, 1964.

56. Mancini, G., Carbonnara, A.O. and Hermans, J.F. Immunochemical quantification of antigens by single radial immunodiffusion, Immunochemistry, 2, 235, 1965.

57. Momma, K. Immunochemical semi-quantitative estimation of gamma M and gamma A immunoglobulins in healthy and diseased children. Part 3: immunoglobulin levels in infectious, allergic disease, collagen diseases, and liver cirrhosis. Acta. Pediat. Jap. 711, 1965.

58. Marsden, P.D., Seah, S.K.K., Mott, K.E., Prata, A. and Platt, H. Immunoglobulins in Chagas' disease. J. Trop. Med. Hyg. 73, 157, 1970.

59. Matteren, P., Mosseyeff, R., Michael, R. and Peretti, P. La Beta gamma-macroglobuline dans la trypanosomaiase. Ann. Inst. Pasteur. 101, 596, 1961.

60. McFarlane, H. Immunoglobulins in Nigerians. Lancet, ii,

62. McGregor, I.A. Immunology of Malarial infection and its possible Consequences. Brit. Med. Bull. 28, 22, 1972.

63. Messeyeff, R.F. and Zisswiller, M.C. A varsatile method of Rodial Immunodiffusion assey imploying microquantities of antiserum. Anal. Biochem. 30, 180, 1969.

64. Michaux, J.L., Les immunoglobulins des Bantous a l'etat normal et pathologique. Ann. Soc. Belge Med. Trop. 46, 491, 1966.

65. Milner, L.V. and Caltiz, F. Serum immunoglobulin levels in Whites, Asiatic and Bantu Blood donors. S. Afr. Med. J. 45, 683, 1971.

66. Rhodes, K., Scott, Marthan, R.L., and Monk-Jones, M.E., Immunological sex differences, Ann. Rheum. Dis. 28, 104, 1969.

67. Ritchie, L.S. An ether sedimentation technique for routine stool examination. Bull. U.S. Army Med. Dept. 8, 444, 1948.

68. Roitt, I.M. Essential Immunology, 3rd edn. Blackwell Scientific Publications, Oxford, 1977.

70. Shulman, G. and Gilich, G. Serum Immunoglobulins in Black South African Children. S. Afr. Med. J. 50, 1465, 1976.

71. Shulmann, G., Gilich, G. and Andrew, M.J.A. Serum Immunoglobulins G, A and M in white and Black adults on the Witwatersrand - S. Afr. Med. J. 49, 1100, 1975.

72. Shedecor, G.W. and Cochran, W.G. Statistical Methods, 6th edn. Oxford and IBM, New Delhi.

73. Soothill, J.F. Estimation of eight serum proteins by gel diffusion precipitin technique. J. Lab. Clin. Med. 59, 859, 1962.

74. Stiehm, E.R. and Fudenberg, H.H. Serum levels of immunoglobulins in health and disease. A survey. Pediatrics, 37, 715, 1966.

75. Targett, G.A.T. Antibody response to Plasmodium falciparum malaria: comparisons of immunoglobulin concentrations, antibody titers and the antiginecity of different asexual forms of the parasite. Clin. Exp. Immunol. 7, 501, 1970.

76. Tabie, J.E., Abele, D.C., Hill G.J., Contacos, P.G. and Evans C.B. Fluorescent antibody studies on the immune response

77. Turner, M.W. and Voller, A. Studies on the immunoglobulins of Nigerians. I. The immunoglobulin levels of a Nigerian population. J.Trop. Med. Hyg. 69, 99, 1966.

78. Weir, D.M. Immunology: an outline for students of Medicine and Biology 4th edn. E.L.B.S. and Churchill Livingstone, Edinburgh, 1977.

79. Wells, J.V. Serum Immunoglobulin levels in tropical Splenomegaly Syndrome in New Guinea. Clin. Exp. Immunol. 3, 943, 1968.

80. West, C.D., Hong, R. and Holland, N.H. Immunoglobulin levels from newborn period to adulthood and in Immunoglobulin deficiency states. J. Clin. Invest. 41, 2054, 1902.

81. West, C.D., Minrichs, V. and Mikle, N.H. Quantitative determination of the serum globulins beta-2A and beta-2M by immuno-electropheretic analysis. J. Lab. Clin. Med. 58, 137, 1961.

82. WHO committee, Nomenclature for human Immunoglobulins. Bull. World Hlth. Org. 30, 447, 1964.

83. William, A.I.O. and McFarlane, H. Distribution of malarial antibody in maternal and cord sera. Arch. Dis. Childhood 44, 511, 1969.

84. Wright, D.J.M. Doniach, D., Less of, M.H., Turk, J.L., Grimble

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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