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Total serum IgE levels in Venezuelan schoolchildren

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Summary

Seventy-eight disease-free children were evaluated by PRIST for total serum IgE in order to establish the normal range for this immunoglobulin and assess its utility, in tropical climates, such as Venezuela, as a diagnostic tool for clinical allergy. Seventy-eight normals were selected from a group of 1053 children, aged 7–12 years from fourteen schools in Caracas. Exclusion from the normal pool was based on nationality, and on historical, clinical, and/or laboratory evidence of atopic and/or infectious diseases, particularly with parasites. In addition to a routine CBC and differential, the following studies were performed: a search for stool ova and parasites; *in vitro* (RAST) and *in vivo* (skin prick) testing for specific IgE to *Dermatophagoides pteronyssinus*, *Aspergillus fumigatus*, and ragweed. Measurement of antibodies against influenza A and B, adenovirus A_2 , cytomegalovirus, parainfluenza 1 and 3, herpes simplex, respiratory syncytial virus, Coxsackie B_1 to B_6, *Mycoplasma pneumoniae* and Rotavirus was also carried out.

Normal serum IgE levels for disease-free children in the age group studied ranged from 1·7–255 u/ml. The highest average level ($\bar{Y}$: 74 u/ml) occurred at the age of 9 years. These values differed significantly from age-matched control groups of known atopic and helminth-infected children. Thus, once common causes for elevated IgE levels are eliminated, determination of total serum IgE can be utilized as a valuable tool in diagnosis of clinical allergy in countries with tropical climates.

Introduction

Immunoglobulin E (IgE) mediates the immediate hypersensitivity reaction. Thus, the measurement of the total serum IgE is of general interest as a diagnostic tool in clinical

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allergy. For this measurement to be of use, however, levels of this immunoglobulin in control subjects must be known. Since wide variations have been found among different areas of the world (levels being particularly elevated where parasitic infestations are prevalent); previous reports (Nye *et al.*, 1975; Berg & Johansson, 1969; Sears, Chow & Morseth, 1980) have stressed the need to determine control levels of total serum IgE in the population at risk. Recognizing the need to determine control levels of this immunoglobulin to serve as a reference in studying atopics in Venezuela, seventy-eight disease free children aged 7–12 years, native to this country and residing in Caracas were evaluated for total serum IgE.

Material and methods

Population criteria for selection

One thousand and fifty-three children between the ages of 7–12 years from fourteen schools in Caracas were screened for participation in the study. A five-step protocol with seventeen criteria was established. Any child who did not fulfil all the criteria was excluded. The study population was made up of one group each of 7, 8, 9, 10, 11 and 12 year olds. Two additional groups of school children, one atopic and the other with parasitic infestation were studied for comparison.

The normal children were those not excluded on the basis of historical, clinical, and/or laboratory evidence of atopic and/or infectious diseases, particularly with parasites. The following stepwise protocol served as the basis of exclusion.

Historical data. A questionnaire to detect the presence of allergy and infection was administered to parents and school directors of the selected institutions; they were instructed in relation to the criteria. Children with a personal or family (first and second degree relatives) history of allergies, i.e. asthma, hay fever, atopic dermatitis, food hypersensitivity, insect hypersensitivity, and/or hives, etc., and/or infectious diseases (parasitic, viral or bacterial) were excluded from further study.

Physical examination. A general examination to detect atopic and infectious diseases was performed by the investigators. Children with wheezing, hives, allergic or infectious rash were excluded from further study.

Routine blood and stool exams. A CBC, and differential and fresh stool for ova and parasites were performed. Children with either eosinophilia or percentages of lymphocytes over the expected age range and/or evidence of parasites in the stools were excluded.

Total serum IgE evaluation. Sera of non-excluded children were evaluated by PRIST (Salmon, Mackey & Fudenberg, 1969) for total serum IgE. For comparison, PRIST was also performed in a group of twelve children who were selected at random from those previously excluded because of parasitic infestation.

Re-evaluation. Children who were not excluded by initial history, physical examination, and routine blood and stool evaluation but who were found to have total serum IgE levels above the arithmetic mean of those tested were re-evaluated by the original questionnaire and physical examination. In addition, RAST and prick tests were performed to determine the presence of specific IgE to: *Dermatophagoides pteronnysinnus*, *Aspergillus fumigatus* and ragweed. Three serial stool tests for ova and parasites, and antibody viral titers against influenza A and B, adenovirus A_2 and B, Cytomegalovirus, parainfluenza 1 and 3, herpes simplex, respiratory syncytial virus, Coxsackie B_1 to B_6, *Mycoplasma pneumoniae* and Rotavirus were also carried out.

For comparison, a group of twenty-five children were selected at random from those previously excluded because of a positive personal history of allergy and/or genetic allergy background. These twenty-five underwent stool examination for ova and parasites, RAST and prick tests with the same antigens used for the normal group.

Skin testing. Skin prick tests were performed on the mid-upper forearms, applying a drop of the test reagent and control solution to sequential pre-marked skin sites on each child selected. Antigen selection included: *Dermatophagoides pteronnysinus*, *Aspergillus fumigatus* and ragweed (short and tall) from Dome Laboratories, U.S.A. Results were read after 15 min, and were graded 0 to 4+ according to previous criteria (Rappaport *et al.*, 1979). Appropriate controls were negative.

Total IgE determination

Blood samples were collected and sera were assayed for total IgE content by the paper radioimmunosorbent test PRIST, from Pharmacia Diagnostics, Sweden (Gleich, Averbeck & Svedlund, 1971). Briefly, the 100 μl 1:10 diluted serum was incubated with an anti-IgE covalently coupled to the paper disc; after washing and a second incubation, a fixed amount of immunosorbent purified anti-IgE ^{125}I was added. Radioactivity was measured in a gamma counter; IgE levels were obtained from the standard curve. Sera from atopic and parasitic children were diluted 1/50.

RAST determination

Radioallergosorbent test (RAST), from Pharmacia Diagnostics, was performed using the following antigens: *Dermatophagoides pteronnysinus*, *Aspergillus fumigatus*, and ragweed (common, western, giant and false). The procedure followed the available commercial kit instructions; results were referred to the standard reference curve (Wide, Bennich & Johansson, 1967).

Viral antibody titers

All the serum samples were used at dilutions of 1:8 or 1:10 depending on the assay employed. To check the antibody levels against the viral agents, the following techniques were used: (1) Haemagglutination inhibition for the two mutants of influenza virus (A_2 Victoria and B Hong Kong) with antigens obtained from the C.D.C. Atlanta (Immunology Series No. 6. Center for Disease Control, 1975); (2) complement fixation test, for the antigens: adenovirus, Cytomegalovirus, parainfluenza 1 and 3, herpes simplex, respiratory sincytial and rotavirus (Casey, 1968); (3) neutralization test in HEp-2 cell cultures for enterovirus Coxsackie B_1 to B_6, employing 100 $TCID_{50}$ of the reference virus strains against two-fold dilutions of each serum (Center for Disease Control, 1974); (4) to evaluate antibodies against *Mycoplasma pneumoniae* the metabolic inhibition test was used utilizing the reference strains of the organism, kindly provided by Dr D. Taylor Robinson (Clinical Research Centre, Middlesex, England) (Taylor *et al.*, 1966).

Statistical analysis

Geometric mean, +1 and +2 s.d. were calculated for each age group. Transformation of each value to natural logarithm was performed to facilitate the analysis of the data.

Results

A total of 1053 school children were initially screened. Only 588 (56%) correctly fulfilled the first step questionnaire, 303 (67·5%) out of the 588 were excluded due to the

following: 240 (40·8%) were clinically atopic; 63 (10·7%) had positive family history of atopy and absence of infectious diseases. A group of fifty-two children (9·2%) had infections and forty-seven (8%) were foreigners. One hundred and eighty-six children were then subjected to the second step; none were excluded, and passed to the third step; 100 were then discharged because of: seventeen with lymphocytosis, twenty-two with parasitic infections (by serial stool examination) and/or eosinophilia; and sixty-one who dropped out of the study. This left a total of eighty-six children for step four; when arithmetic means by ages were calculated, forty-three of them showed levels of IgE within the selected range, but above the mean; this latter group was further screened by subjecting them to step five, where viral, parasitic, and atopic diseases were ruled out. Eight children were excluded from the study due to the presence of specific IgE detected either by skin prick test or RAST to the antigens searched for, in the absence of clinical manifestations. These same eight children underwent clinical and laboratory tests for an additional period of 12 months. This investigation will be the subject of a separate report.

After the five-step protocol was completed, a total of seventy-eight healthy school children represented the group from which normal total IgE was obtained.

The geometric mean of total IgE levels by age is depicted in Fig. 1, showing a peak value at the age of 9 years; the distribution of the total IgE levels is shown in Fig. 2,

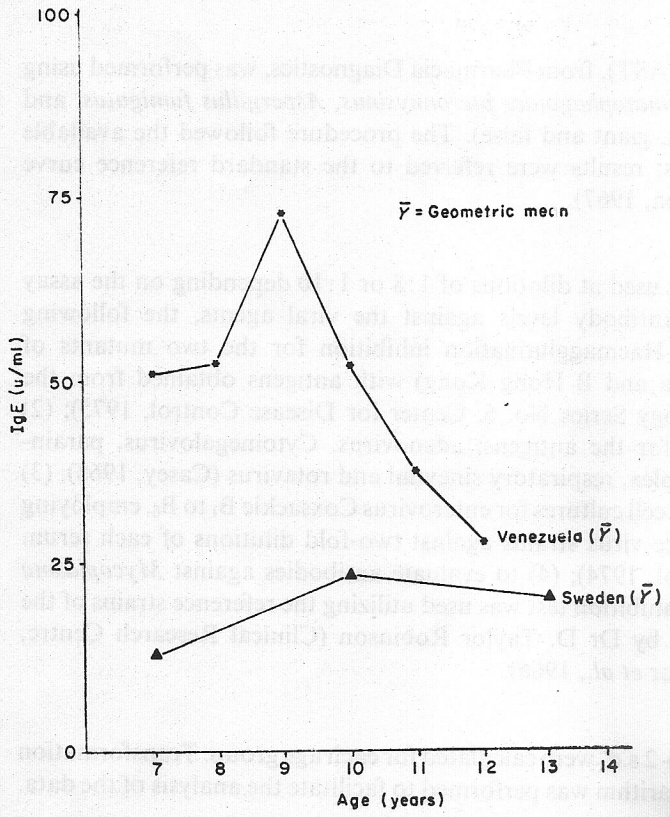

Fig. 1. Total normal serum IgE levels in healthy Venezuelan schoolchildren.

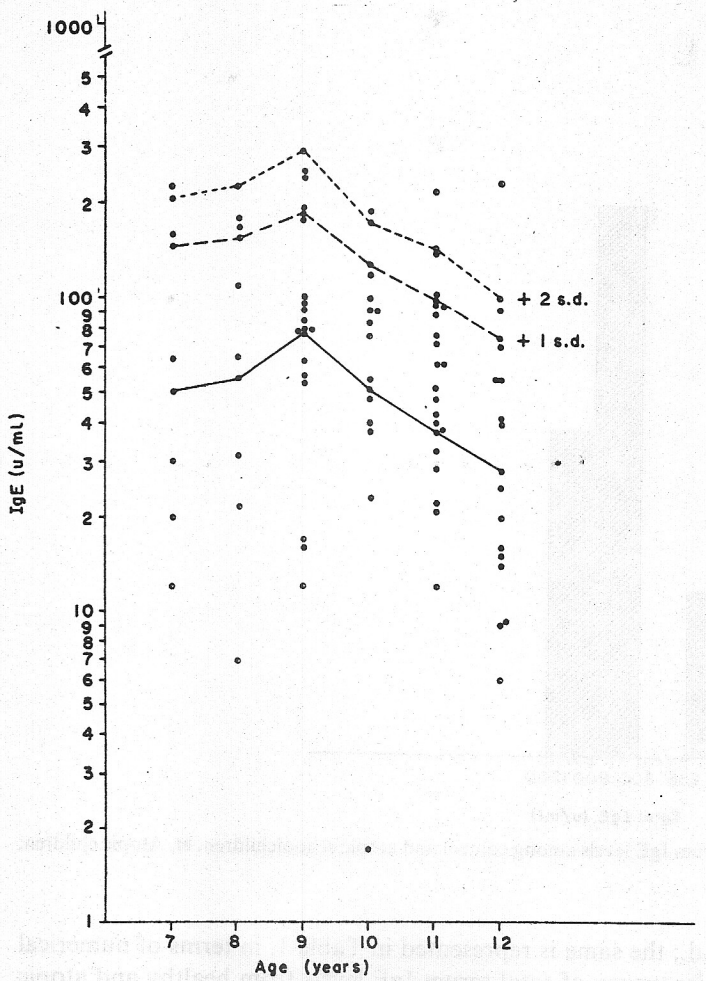

Fig. 2. Distribution of total serum IgE levels in Venezuelan schoolchildren.

Table 1. Normal serum IgE (μ/ml)

Age	n	$\bar{Y}$ (u/ml)	+1 s.d. (u/ml)	+2 s.d. (u/ml)
7	6	51	147	208
8	7	53	155	226
9	17	74	176	297
10	13	52	123	172
11	20	38	100	148
12	15	28	74	100

$\bar{Y}$: Geometric mean.

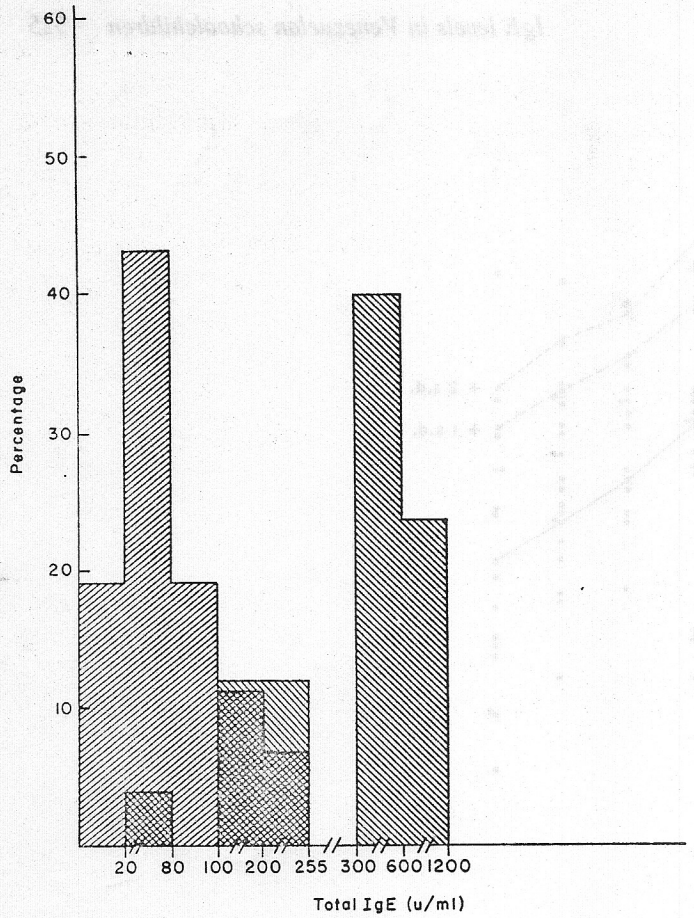

Fig. 3. Distribution of total serum IgE levels among control and atopic schoolchildren. ▧, Atopic children; ▨, healthy children.

including +1 and +2 s.d.; the same is represented in Table 1, in terms of numerical means. The cumulative frequency of total serum IgE levels from healthy and atopic groups is depicted in Fig. 3, where levels from the normal group are mainly distributed between 21–80 u/ml (range: 1·7–255 u/ml). Sixty-four per cent of the atopic non-parasitic group, had total serum IgE levels over 300 u/ml. Forty-four percent suffered from a mixed type of atopic disease mainly constituted by extrinsic bronchial asthma, and seasonal allergic rhinitis. A geometric mean of 345 u/ml was found among this randomly selected atopic group.

The parasitic non-atopic group of children screened by serial stool tests, showed tricocephalus, guiardia lamblia, and chilomastix mesnini infestations. Only those three children with helminths showed IgE levels above 300 u/ml.

Discussion

Many attempts have been made to establish normal IgE levels in healthy populations at risk, both in children and adult groups. Such efforts have been hampered in developing areas of the world, by the overwhelming prevalence of parasitic infestation.

In the present study, the step-wise approach utilized by Kjellmann, Johansson and Roth (1976), was modified, adding to the screening the systematic search for parasitic and viral diseases. One thousand and fifty-three schoolchildren from fourteen schools in Caracas were screened and seventy-eight of them fulfilled the criteria to serve as a sample to establish the IgE levels in normal Venezuelan children.

An increase in IgE levels with age was observed, reaching a peak at the age of nine ($\bar{Y}$: 74 u/ml). This peak value is earlier than the observed in the Swedish series; furthermore, within the same range of values, our total geometric mean (from 7–12 years) was 1·7 times higher than that of the Swedish.

There was no significant difference in the IgE levels of males and females, which agrees with previous reports (Grundbacher, 1975; Berg & Johansson, 1969).

Immunoglobulins in general, appear to be higher in members of populations who evolved in the tropics than in the populations which evolved in temperate climates (Grundbacher, 1975); increased IgE levels have been demonstrated in black populations compared with white populations (Grundbacher, 1974), and in viral disease (Parelmutter, Phipps & Potvin, 1978). Johansson, Mellbin and Vahlquist (1968) determined total IgE levels in two groups of Ethiopian children, one was sixteen times higher than European children, and the other twenty times. He also found in children with parasitic infestation (*Ascaris lumbricoides*) from the same area, total IgE levels twenty-eight times higher than the Swedish series.

Contrary to the above mentioned data, our results obtained from normal healthy children suggest that IgE levels are not necessarily elevated in developing areas of the world but may reflect the role played by genetic background and its adaptation to local environmental factors.

Several authors (Ishizaka & Ishizaka, 1976; Orgel *et al.*, 1975; Hamburger *et al.*, 1974; Kjellman, 1976; Johansson, 1968; Gerrand *et al.*, 1974), have stressed the usefulness of establishing normal IgE levels in the healthy population as a diagnostic tool in clinical allergy or as a predictive tool when screening groups of newborns or of children.

Finally, there is no clear explanation as to why normal IgE levels from Venezuelan School children peak earlier than the Swedish. It might be possible to speculate in both instances, that the mechanism controlling IgE synthesis may either be perturbed as suggested by Katz (1978), in the murine model or else play a distinct useful role in this age group.

Once helminthic infestation is ruled out, an elevated level of IgE is highly suggestive of allergy a cause of significative morbidity in developing areas of the world.

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References

CENTER FOR DISEASE CONTROL (1975) *Advanced laboratory techniques for influenza diagnosis, Immunology series No. 6.* U.S. Department of Health, Education and Welfare, Public Health Service, Atlanta, U.S.A.

BERG, T. & JOHANSSON, S.G.O. (1969) Immunoglobulins levels during childhood, with special regard to IgE. *Acta Paediatrica Scandinavia*, **58,** 513.

CASEY, H.L. (1968) *Standardized diagnostic complement fixation: method and adaptation to microtest. Public Health Monograph 74.* Center for Disease Control, Atlanta, Georgia, U.S.A.

GERRAND, J.W., HORNE, S., VICKERS, P., MACLENZIE, J.W., GOLUBOFF, N., GARSON, J.Z. & MANINGGS, C.S. (1974) Serum IgE levels in parents and children. *Journal of Pediatrics*, **85,** 660.

GLEICH, G.J., AVERBECK, A.K. & SVEDLUND, H.A. (1971) Measurement of IgE in normal and allergic serum by radioimmunoassay. *Journal of Laboratory and Clinical Medicine*, **77,** 690.

GRUNDBACHER, F.J. (1974) Heritability estimates and genetic and environmental correlations for the human immunoglobulin G, M, and A. *American Journal of Human Genetics*, **26,** 1.

GRUNDBACHER, F.J. (1975) Causes of variation in serum IgE levels in normal populations. *Journal of Allergy and Clinical Immunology*, **56,** 104.

HAMBURGER, N.R., LENOIR, M., GROSHONG, T.E., RANDALL, J., WALLACE, W.M. & ORGEL, H.A. (1974) Development of IgE and allergy during the first year of life; Preliminary data. *Journal of Allergy and Clinical Immunology*, **53,** 94.

ISHIZAKA, K. & ISHIZAKA, T. (1976) Immunoglobulin E. Current Status and clinical laboratory applications. *Archives of Pathology and Laboratory Medicine*, **100,** 289.

JOHANSSON, S.G., MELLBIN, T. & VAHLQUIST, B. (1968) Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND). *Lancet*, **i,** 1118.

JOHANSSON, S.G. (1968) Serum, IgND levels in healthy children and adults. *International Archives of Allergy*, **34,** 1.

KATZ, D. (1978) Control of IgE antibody production by suppressor substances. *Journal of Allergy and Clinical Immunology*, **62,** 44.

KJELLMAN, N.I., JOHANSSON, S.G. & ROTH, A. (1976) Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). *Clinical Allergy*, **6,** 51.

KJELLMAN, N.I. (1976) Predictive value of high IgE levels in children. *Acta Pediatrica Scandinavica*, **64,** 465.

CENTER FOR DISEASE CONTROL (1974) Neutralization test for enterovirus in cell culture (1974) In: *Laboratory Diagnosis of Viral Diseases, Course 8241-C.* p. 98. Center for Disease Control, Atlanta, Georgia, U.S.A.

NYE, L., MERRET, T.G., LANDON, J. & WHITE, R.J. (1975) A detailed investigation of circulating IgE levels in a normal population. *Clinical Allergy*, **6,** 13.

ORGEL, H.A., HAMBURGER, R.N., BAZARAL, M., GORRIN, H., GROSHONG, T., LENOIR, M., MILLER, J.R. & WALLACE, W. (1975) Development of IgE and allergy in infancy. *Journal of Allergy and Clinical Immunology*, **56,** 296.

PALERMUTTER, L., PHIPPS, P. & POTVIN, L. (1978) Viral infections and IgE levels. *Annals of Allergy*, **41,** 158.

RAPPAPORT, I., PONCE, D., SONG, D. & WANG, Y. (1979) On the correlation between Rast and the allergy intradermal test. *Annals of Allergy*, **43,** 1.

SALMON, S.E., MACKEY, G. & FUDENBERG, H.H. (1969) 'Sandwich' solid phase radioimmunoassay for the quantitative determination of human immunoglobulins. *Journal of Immunology*, **103,** 129.

SEARS, M.R., CHOW, C.M. & MORSETH, D.J. (1980) Serum total IgE in normal subjects and the influence of a family history of allergy. *Clinical Allergy*, **10,** 423.

TAYLOR, R.D., PURCELL, R.H., WONG, D.C. & CHANOCK, R.M. (1966) A coulor test for the measurement of antibody to certain mycoplasma species based upon the inhibition of acid production. *Journal of Hygiene (Cambridge)*, **64,** 91.

WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet*, **ii,** 1105.