Immunoglobulin values in healthy Thai children aged ≤ 24 months determined by nephelometry

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Summary

Background: **Variation of normal immunoglobulin (Ig) levels between different genetic and environment factors has been studied. Although antibody deficiency diseases can start from infancy, data of Ig reference levels in children aged ≤24 months are still limited, especially in Asian children.**

Purpose: **The aim of this study was to determine serum IgG, IgA, IgM, and IgG subclasses in healthy Thai children from the newborn period to age 24 months.**

Methods: **Serum samples were collected from healthy Thai children age <1-24 months to measured serum IgG, IgA, IgM, and IgG subclasses by nephelometry.**

Results: **Of the 100 infants, 44% were female with a median (range) age of 13 (0.3-24) months. The geometric mean IgG was 803 mg/dL, IgA 36 mg/dL, and IgM 102 mg/dL. The mean IgG1 was 646 mg/dL, IgG2 127 mg/dL, IgG3 45 mg/dL, and IgG4 17 mg/dL. The average ratios of IgG subclass 1:2:3:4 were 77:15:6:2%. No significant differences in each immunoglobulin isotype between genders were found. Our mean IgG level was slightly lower than that in healthy Thai children, measured by radial diffusion method but not significant except 1-3 months (p = 0.016). However, the mean IgG level in our study was higher than that reported by radial diffusion in healthy US children (p <0.001).**

Conclusions: **This study illustrated the importance of having normal Ig values from age- and ethnically-matched controls by high precision nephelometric assay in order to appropriately diagnose immunologic disorders in Asian infants.** ***(Asian Pac J Allergy Immunol 2013;31:307-13)***

Key words: *children, immunoglobulin level, immunodeficiency, infants, nephelometry*

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Submitted date: 1/10/2012

Accepted date: 17/1/2013

Introduction

Antibody deficiency is the most common category of primary immunodeficiency accounting for more than 50% of all primary immunodeficiency diseases (PID).[1] The onset of severe antibody deficiency disease starts in infancy so early diagnosis and treatment are keys to better prognosis.[2] Serum immunoglobulin (IgG, IgA, IgM, and IgG subclasses) concentrations are used to evaluate immune status and diagnose PID.[3] A new classification of PID has been defined recently,[4, 5] so precise age-specific normal immunoglobulin values is very important.

Genetic and environmental factors may produce variation in Ig levels. Ig levels in Thai children, using the radial immunodiffusion assay, showed higher levels of IgG, IgA, IgM in Thai children compared to those in Western countries.[6-8] Our team reported the significant differences of

geometric mean IgG concentrations between Thai and Caucasian children and adolescents.[9] The IgG levels of Thai children aged 2-15 years[9] were higher than the levels of American[10] and Turkish children.[11] Geography, nutrition, and socioeconomic status are environmental factors that affect the incidence of infectious diseases and may cause differences in serum Ig levels. For instance, children living in the USA and Turkey, where people have better socioeconomic status than Thai people, have lower Ig levels. The improvement of sanitization has reduced community acquired infections. This may influence the decreasing serum Ig levels of Thai children at the present time compared to studies done over the last 4 decades.[6-8, 12, 13]

Most primary immunodeficiency diseases start in childhood meaning that standardized normal values of IgG, A, M and IgG subclasses in children are very important. The method of assay may also affect the Ig levels. Previous studies used the radial immunodiffusion method of Mancini,[14] but a new method called nephelometry has recently been used globally.[11,15-17] Even though there are studies measuring serum Ig levels by nephelometry in Western countries, to our knowledge, only our recently published article reported the results in Asian children aged 2-15 years.[9] For this study, we aim to evaluate Ig values in healthy Thai infants because normal levels differ so much in these ages and there is still limited data from which reference levels can be derived.

Methods

Serum samples were collected from healthy Thai children age <1-24 months from the well child clinic of King Chulalongkorn Memorial Hospital, Chulalongkorn University in Bangkok, Thailand as part of the HIV-NAT 108 study. Exclusion criteria were children with abnormal growth (below 3rd or above 97th percentile, according to the Thai growth chart), febrile illness, respiratory and other infections at screening, or medical illnesses that might result in abnormal immunity such as HIV infection or exposure, allergic conditions, and children who used anti-infective agents or corticosteroids in the past month. Four ml of blood obtained by venipuncture was collected, centrifuged for serum, and frozen at -70 °C at the HIV-NAT laboratory in Bangkok and sent to Ramathibodi Hospital laboratory under controlled conditions for serum Ig measurement. The quantifications of IgG, IgA, IgM, IgG1, IgG2, IgG3, and IgG4 levels were measured on the Nephelometer Analyzer and commercially available using reagents and an automated system (SIEMENS) by nephelometric method. The coefficients of variations (CV) of intra and inter assay were less than 5%.

This study was approved by the institutional review boards of Chulalongkorn University. All caregivers gave consents.

Statistical analyses

Median, interquartile range (IQR) or frequency percentage distributions were calculated according to the type of variables for demographic data. Immunoglobulin values were summarized according to the age groups by using the geometric mean and 95% confidence interval (95% CI). According to their age, the children were categorized into 5 groups: 0-2, 2-6, 6-12, 12-18, and 18-24 months old. T-test or ANOVA techniques, as appropriate, were used for comparing differences of Ig values according to gender and age groups, and comparing IgG data from this study to published data from Thailand and other countries. Linear regression analysis was used to assess factors associated with increasing IgG including gender and age. Log transformation was used to improve normalization and was anti-logged back to geometric ratio for presentation. If it could not improve normalization by log transformation, a nonparametric test was applied. In all analyses, effect sizes and 95% confidence intervals around this effect size were given in addition to *p* values.

Results

One hundred children, 44% female, were enrolled. The median age was 13 months. The age range was 0.3 to 24 months. There were 2 children aged < 1month (0.3 and 0.6 months) in 0-2 month age group. Children were categorized according to age into 0-2, 2-6, 6-12, 12-18, and 18-24 months old (Table 1). Serum IgG, IgA, IgM and IgG1, IgG2, IgG3, and IgG4 concentrations classified by ages are demonstrated in Table 2. Overall, the geometric mean (SD) IgG was 803 (289) mg/dL, IgA was 36 (28) mg/dL, IgM was 102 (45) mg/dL. The mean serum IgG level was 774 mg/dL in girls and 827 mg/dL in boys (p =0.332). For IgG subclasses, the mean (SD) IgG1 was 646 (239) mg/dL, IgG2 was 127 (45) mg/dL, IgG3 was 45 (63) mg/dL, and IgG4 was 17 (34) mg/dL. No significant difference in each immunoglobulin isotypes between genders was found. The average ratios of IgG subclasses for IgG1:IgG2:IgG3:IgG4 were 77: 15: 6: 2%.

Table 1. Characteristics of children

Values*	N=100
Age, median (IQR), months	13 (6.7-18.5)
% female	44
Weight (kg), median (IQR)	10 (7,11)
Length (cm), median (IQR)	74 (65, 80)
Number (%) of children in each age group in month, n(%)	
0-2	8(8.00)
2-6	16(16.00)
6-12	24(24.00)
12-18	25(25.00)
18-24	27(27.00)

*Data are presented as median (IQR)

Figure 1 shows mean (SD) of Ig levels in different age groups. The IgG levels increased rapidly between 2-12 months of age, followed by a slow increase during 12-18 months and stable levels between 18-24 months. The IgA levels were similar in all age groups. The IgM levels rose gradually from 0-6 months and did not change with increasing age until 24 months.

The mean IgG subclass levels in different age groups were shown in Figure 2. The IgG1 levels increased rapidly between aged 2-12 months; then, gradually increased during 12-18 months and appeared to plateau like the pattern for total IgG. IgG2, IgG3 and IgG4 levels were similar across age groups.

By sub-analysis, we compared IgG levels in our data with 2 previous studies performed in Thai and in US children, which measured Ig by radial immunodiffusion (RID) method. Firstly, data were compared with the previous study from Thai children,[8] IgG levels in all age groups from our study were lower than those in the previous Thai study but it was non-statistically significant (p =0.31). For IgA, levels in all age groups in our study were lower than the previous Thai study with statistical significance in 2 age groups; 7-9 months (p =0.028) and 10-18 months (p =0.002). IgM levels from our study were higher than the previous study in all age groups with statistically significant differences in 4-6 months (p =0.020) and 10-18 months (p =0.025) (Table 3).

Secondly, we compared our data with another study from the US by Stiehm et al.[10] which has been used as reference values in Thailand; geometric means of IgG in every age group from our study were significantly higher than those in the US study

Table 2. Immunoglobulin levels by age groups

Age (months)	N	Geometric mean (mg/dl)	SD	minimum	maximum	95%CI
IgG						
0-2	8	676	211	459	1170	(539,847)
2-6	16	530	128	377	774	(469,599)
6-12	24	771	316	363	1690	(661,899)
12-18	25	964	223	666	1340	(878,1058)
18-24	27	947	250	653	1880	(868,1034)
IgA						
0-2	8	9	8	6	27	(5,14)
2-6	16	24	12	13	56	(19,30)
6-12	24	36	17	7	78	(29,44)
12-18	25	53	28	24	116	(44,65)
18-24	27	47	31	20	149	(39,58)
IgM						
0-2	8	44	27	24	111	(30,65)
2-6	16	82	33	40	141	(66,102)
6-12	24	103	41	48	249	(90,119)
12-18	25	121	41	76	233	(107,137)
18-24	27	126	42	77	234	(112,143)
IgG1						
0-2	8	496	193	267	922	(367,671)
2-6	16	431	111	290	669	(378,491)
6-12	24	646	259	299	1400	(556,751)
12-18	25	779	196	466	1130	(703,863)
18-24	27	749	201	491	1460	(683,821)
IgG2						
0-2	8	143	18	117	171	(128,160)
2-6	16	103	36	59	202	(87,121)
6-12	24	108	43	66	251	(94,125)
12-18	25	139	29	101	236	(128,151)
18-24	27	149	55	54	284	(128,173)
IgG3						
0-2	8	23	9	12	40	(17,32)
2-6	16	38	35	18	146	(27,53)
6-12	24	51	25	22	105	(42,61)
12-18	25	56	116	20	624	(43,73)
18-24	27	46	26	10	136	(37,56)
IgG4						
0-2	8	31	26	4	73	(13,74)
2-6	16	11	9	1	33	(7,18)
6-12	24	10	34	1	166	(6,17)
12-18	25	15	21	2	80	(10,23)
18-24	27	33	44	3	190	(22,48)

Ig; immunoglobulin, SD; standard deviation, 95% CI; 95% confidence interval

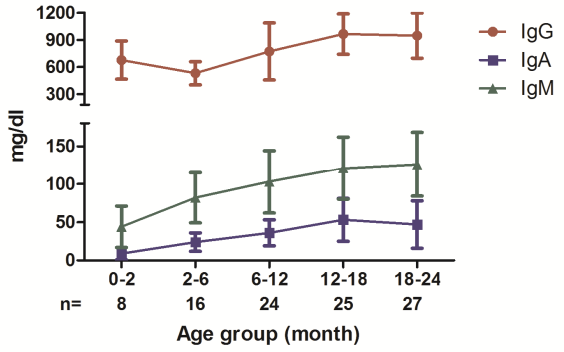

Figure 1. IgG, A and M levels in the different age groups (Means and SD)**.** The IgG levels increased rapidly between 2-12 months of age, followed by a slow increase during 12-18 months and stable levels between 18-24 months. The IgA levels were similar in all age groups. The IgM levels rose gradually from 0-6 months and did not change with increasing age until 24 months.

in every age group (p <0.001). For IgA, the levels from our study were not statistically different from the US study and IgM levels from our study were higher with statistically significant differences in 2-6 month (p <0.001), 6-12 month (p =0.002) and 12-24 month (p <0.001) groups (Table 3).

In addition, we demonstrated the comparison of geometric means of IgG between our study and a Turkish study which is the only published data available using nephelometry.[11] IgG levels of young Thai children were higher than Turkish children for all age groups but there was a significant difference only in the 12-24 month group (p <0.001). IgA levels from our study were higher than the Turkish study with a significant difference in <1-6 month (p =0.032) while IgM levels from our study were higher with significant differences in <1-6 month and 12-24 month groups (p =0.001) (Table 4).

Discussion

Our study illustrates the importance of having normal Ig values from age- and ethnically-matched controls by high precision nephelometric assay in order to diagnose early immunologic disorders in

Table 3. Comparison of Ig levels of Thai children in this study with previous Thai study*[8] and US study*

Age (months)	Thai Geometric mean(95%CI)	Thai * (Thongchareon) Geometric mean(95%CI)	US* Geometric mean(95%CI)	P-value**	P-value***
IgG					
1-2	676(539,847) N=8	845(760,930) N=52	428(404,454) N=100	0.016	<0.001
2-6	530(469,599) N=16	703(601,805) N=36	370(355,387) N=200	0.116	<0.001
6-12	771(661,899) N=24	971(783,1159) N=18	531(497,568) N=100	0.064	<0.001
12-24	955(898,1016) N=52	1113(919,1307) N=18	682(646,720) N=100	0.086	<0.001
IgA					
1-2	9(5,14) N=8	20(15, 25) N=52	14(12,16) N=100	0.455	0.109
2-6	24(19,30) N=16	35(27, 43) N=36	23(22,26) N=200	0.383	0.881
6-12	36(29,44) N=24	64(47, 81) N=18	38(35,42) N=100	0.028	0.519
12-24	50(44,57) N=52	78(64, 92) N=18	78(71,86) N=100	0.002	0.297
IgM					
1-2	44(30,65) N=8	49(40,58) N=52	45(42,49) N=100	0.480	0.881
2-6	82(66,102) N=16	73(61,85) N=36	57(54,59) N=200	0.020	<0.001
6-12	103(90,119) N=24	82(67,97) N=18	81(76, 86) N=100	0.103	0.002
12-24	124(114,135) N=52	95(78, 112) N=18	94(88,100) N=100	0.025	<0.001

* radial immunodiffusion method
**P-value compared Thai children in this study with previous Thai study[8]
***P-value compared Thai children in this study with US study[10]
Ig; immunoglobulin, 95% CI; 95% confidence interval

Table 4. Comparison of Ig measured by nephelometry between Thai children in this study and Turkish children[11]

Age (months)	Thai Geometric mean(95%CI)	Turkish Geometric mean(95%CI)	P-value
IgG			
<1-6	575(514,643) N=24	677(519,883) N=28	0.289
6-12	771(661,899) N=24	645(558,746) N=44	0.109
12-24	955(898,1016) N=52	774 (748,851) N=60	<0.001
IgA			
<1-6	17(13,23) N=24	10(6, 15) N=28	0.032
6-12	36(29,44) N=24	39(27,57) N=41	0.713
12-24	50(44,57) N=52	44 (43,53) N=57	0.095
IgM			
<1-6	67(54,83) N=24	30(20, 46) N=25	0.001
6-12	103(90,119) N=24	79(63,99) N=44	0.125
12-24	124(114,135) N=52	98(96,118) N=57	0.001

Ig; immunoglobulin, 95% CI; 95% confidence interval

Asian infants. Serum IgG levels decrease rapidly after birth to lower levels at 2-6 months. There is some synthesis of IgG during months 1-6 and the maternal IgG from placenta decreases until it disappears at month 6.[3] The IgG values increase rapidly from 6 months to 2 years old; after that, the rate of increase slows and reaches a plateau by 8-10 years. Serum IgM levels gradually increase from birth and reach adult levels by one year. Serum IgA levels increase slowly and reach adult levels at 10 years.[10,18] Results from our study show similar development of IgG, IgA, IgM to the previously mentioned studies (Figure 1).

For serum IgG subclass concentrations in normal children, the IgG1 values in infants are high during months 0-2 and decrease during months 2-6. These follow the same pattern as total IgG. Serum IgG2, IgG3, and IgG4 levels increase gradually with age especially after 2 months of life. Our results correlated well with previous findings in 1-24 month old children.[11,19] One study reported that IgG1 concentrations were close to adult levels at 8 months old, and IgG3 levels rose to adult levels at 3 months old. IgG2 and IgG4 levels did not plateau at 2 years.[20] This difference may be due to a small sample size; they only had data from 38 adults and 95 infants up to 2 years with no values from older children.

Ethnicity is an important factor in measuring normal Ig levels. A previous study compared IgG subclasses in black and white children and revealed significant differences. IgG2 and IgG4 levels in black children were lower than white children older than 18 months.[21] The comparison of mean IgG values in our study and the study done by Stiehm et al.[10] showed, in Table 3, that Thai children during infancy to 2 years had significantly higher IgG levels than American children. Geometric means of IgG from our study were also higher than a study in Turkey[11] in most age groups with significant differences in only 12-24 months, as shown in Table 4.

One possible cause of non-significant differences of Ig levels could be due to the method used in the study. Aksu et al reported Ig levels by nephelometry,[11] the same method as our study. The study in the USA[10] used the RID technique. Even though there is no agreement on the most sensitive and specific method to measure serum Ig, nephelometric assay has recently been used in many countries. The nephelometry method is easy, rapid, reproducible, and precise[20,22] while the RID technique needs 72 hours for interpreting. Another problem of the RID technique is IgG4 measurement. Previous studies revealed IgG4 values of children were broad and the normal ranges could not be established.[2,21] By contrast, studies done by the nephelometric technique such as our study are able to establish both geometric mean and normal ranges of IgG4 in every age group.

Our results show that trends of Ig levels in Thai infants are lower than previous studies using radial immunodiffusion assay. [6-8] The comparison of IgG levels in this study and a previous study was shown in Table 3 and revealed a trend for decreasing IgG levels in our study.[8] One possible explanation is health care development decreasing rates of infectious diseases. The method of measurement may have also influenced the Ig values as the previous Thai studies used the RID method. To our knowledge, there is no study in Thailand apart from ours which has used the nephelometric method.

This study tried to exclude the subjects who had infection prior to screening by calling all parents one week before enrollment to make sure that children

were healthy and inviting children to come only if they were still healthy on the enrollment day. Complete physical examination was done by pediatricians on enrollment day to ensure that the subjects were healthy. However, no striking elevation of total IgG in individual subject implied that there was no subject who had infection just prior to enrollment.

Our study limitation is that the number of healthy infants especially in younger age groups was lower compared to previous reports in Thai and US children.[8, 10] However, the 95% CI of IgG levels in each age group are quite narrow, indicating that there were not many variations among individuals. Therefore, data from larger sample size may not be different from this study. Further studies with larger numbers of children at younger age groups with different genetic and environmental factors would be useful. In addition, studies should determine the pathophysiology of these differences. In conclusion, it is important to diagnose PID using accurate standard references for Ig values that are matched for age, ethnicity, environmental factors, and method of measurement.

Acknowledgements

We are grateful to the children and their families for participating in this study. This study was supported by the Thailand Research Fund (TRF, grant number BRG5280017) and the Faculty of Tropical Medicine, Mahidol University. We thank Amanda Elisabetta Clarke, MD for proof-reading the manuscript.

The HIV-NAT 108 Study Group includedfrom HIV-NAT: Wasana Prasitsuebsai, Oratai Butterworth, Thongsuai Chuanjaroen, Tawan Mengthaisong, Sasiwimol Ubolyam, Apicha Mahanontharit, Phonethipsavahn Nounthong, Channuwat Bouko, Tanyathip Jaimulwong, Bunruan Sopa, Stephen Kerr. The Pediatric Infectious Diseases Unit, Chulalongkorn University Hospital: Arene Klinklom, Chareeya Thanee, Nichada Naknoi, Napasri Kuljarusiri, Areeratana Piromwong.

References

1. Bonilla FA, Bernstein IL, Khan DA, Ballas ZK, Chinen J, Frank MM, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. Ann Allergy Asthma Immunol. 2005;94(5 Suppl 1):S1-63.
2. Ballow M. Primary immunodeficiency disorders: antibody deficiency. J Allergy Clin Immunol. 2002;109:581-91.
3. Stiehm ER, Ochs HD, Winkelstein JA. Antibody Deficiencies. In: Ochs HD, Winkelstein JA, Stiehm ER, editors. Immunologic Disorders in Infants & Children. Philadelphia: Saunders; 2004. p. 357-369.
4. Notarangelo LD, Fischer A, Geha RS, Casanova JL, Chapel H, Conley ME, et al. Primary immunodeficiencies: 2009 update. J Allergy Clin Immunol. 2009;124:1161-78.
5. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S182-94.
6. Sakulramrung R, Chumdermpadetsuk S, Ngamphaoboon J, Hanvivatvongs O, Vacharasriksorn A, Likitnukul S. A study of serum immunoglobulin levels in Thai children. Chula Med J. 1998;32:233-42.
7. Sirisinha S, Charupatana C, Chitinand S, Petchclai B. The development of serum immunoglobulin levels in the Thais. J Med Assoc Thai. 1970;53:387-96.
8. Thongcharoen P, Wasi C, Panpatana P, Sarasombath S. Determination of human serum immunoglobulins in healthy Thai subjects. J Med Assoc Thai. 1972;55:347-55.
9. Sitcharungsi R, Ananworanich J, Vilaiyuk S, Apornpong T, Bunupuradah T, Pornvoranunt A, et al. Nephelometry determined serum immunoglobulin isotypes in healthy Thai children aged 2-15 years. Microbiol Immunol. 2012;56:117-22.
10. Stiehm ER, Fudenberg HH. Serum levels of immune globulins in health and disease: a survey. Pediatrics. 1966;37:715-27.
11. Aksu G, Genel F, Koturoglu G, Kurugol Z, Kutukculer N. Serum immunoglobulin (IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique. Turk J Pediatr. 2006;48:19-24.
12. Ngamphaiboon J, Theamboonlert A, Poovorawan Y. Serum IgG subclass levels in a group of healthy Thai children. Asian Pac J Allergy Immunol. 1998;16:49-55.
13. Vichyanond P, Petranand S, Senawong S, Banchuen N, Assateerawat A, Tuchinda M. Normal IgG subclass and immunoglobulin levels in Thai school children. Thai J Pediatr. 1996;35:233-8.
14. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry. 1965;2:235-54.
15. Cuilliere ML, Montagne P, Bessou T, Omari R, Riochet D, Varcin P, et al. Microparticle-enhanced nephelometric immunoassay (Nephelia) for immunoglobulins G, A, and M. Clin Chem. 1991;37:20-5.
16. Isaacs D, Altman DG, Tidmarsh CE, Valman HB, Webster AD. Serum immunoglobulin concentrations in preschool children measured by laser nephelometry: reference ranges for IgG, IgA, IgM. J Clin Pathol. 1983;36:1193-6.
17. Pressac M, Allouche F, Circaud R, Aymard P. Evaluation of human IgG subclass assays on Beckman array. Ann Clin Biochem. 1995;32:281-8.
18. Belldegrin A, Shoenfeld Y, Pick AI, Vana D. Age related distribution of serum immunoglobulin concentration in 1003 healthy children and adults. Biomedicine. 1980;33:8-12.

19. Gregorek H, Imielska D, Gornicki J, Mikolajewicz J, Przeradzka B, Madalinski K. Development of IgG subclasses in healthy Polish children. Arch Immunol Ther Exp (Warsz). 1994;42:377-82.
20. Morell A, Skvaril F, Hitzig WH, Barandun S. IgG subclasses: development of the serum concentrations in "normal" infants and children. J Pediatr. 1972;80:960-4.
21. Ambrosino DM, Black CM, Plikaytis BD, Reimer CB, Lee MC, Evatt BL, et al. Immunoglobulin G subclass values in healthy black and white children. J Pediatr. 1991;119:875-9.
22. Vlug A, Nieuwenhuys EJ, van Eijk RV, Geertzen HG, van Houte AJ. Nephelometric measurements of human IgG subclasses and their reference ranges. Ann Biol Clin (Paris). 1994;52:561-7.