

## Serum immunoglobulin-G subclasses levels in normal Thai young adults

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*Immunoglobulin-G (IgG) subclasses are increasing in their clinical importance especially in immunodeficiencies, gammopathy, and some allergic diseases. To determine IgG subclass abnormalities, the normal range of serum concentration of each subclass is needed. However, the serum levels of IgG subclasses always vary with age, race and geographic distribution. This is a study of serum concentration of all four IgG subclasses in 31 Thai adults utilizing commercial IgG subclass-specific antisera in the radial immunodiffusion method. The semi-quantitative serum levels were averaged geometrically, the means of IgG1, IgG2, IgG3, and IgG4 being 1,031.4, 476.2, 48.7, and 78.3 mg/dl, respectively. These results showed some difference compared with other studies. However, these might be suitable for determining IgG subclass abnormalities in Thai adults when using the same procedure of measurement.*

*Key words : Immunoglobulin-G subclass, IgG, IgG subclass.*

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ความผิดปกติของระดับ IgG subclass มีบทบาทสำคัญในโรคทางภูมิคุ้มกันต่าง ๆ เช่น โรคภูมิคุ้มกันบกพร่อง โรค gammopathy และโรคภูมิแพ้ การที่จะตัดสินว่าระดับ IgG subclass ที่ตรวจได้นั้นมีระดับผิดปกติหรือไม่ จำเป็นต้องมีค่าปกติสำหรับเปรียบเทียบ ค่าปกติดังกล่าวนี้จะมีค่าไม่แน่นอนขึ้นอยู่กับอายุ เชื้อชาติและถิ่นที่อยู่ของประชากร การศึกษานี้เป็นการศึกษาระดับ IgG subclass ในคนไทยปกติรวมทั้งสิ้น 31 ราย ด้วยวิธี radial immunodiffusion โดยใช้ชุดทดสอบสำเร็จที่มีแอนติซีรัมจำเพาะต่อแต่ละ subclass ของ IgG ระดับ IgG subclass ที่ตรวจวัดได้นำมาหาค่าเฉลี่ยโดยคำนวณมัชฌิมเรขาคณิต พบว่าระดับเฉลี่ยของ IgG1, IgG2, IgG3 และ IgG4 เป็น 1031.4 mg/dl, 476.2 mg/dl, 48.7 mg/dl และ 78.3 mg/dl ตามลำดับ ผลดังกล่าวแตกต่างจากรายงานในต่างประเทศบ้าง อย่างไรก็ตามค่าที่ได้นี้เหมาะสำหรับเป็นค่าอ้างอิงในการวินิจฉัยความผิดปกติของระดับ IgG subclass ในคนไทยโดยเฉพาะอย่างยิ่งเมื่อวัดด้วยวิธีการเดียวกัน

Immunoglobulin-G (IgG) is the major protein involved in humoral immunity. Immunoglobulin-G protects against various infectious agents, particularly bacteria. Immunoglobulin-G can be divided into four subclasses according to the gamma chain of each, the physicochemical characteristics of the subclasses being based largely on studies of myeloma proteins.<sup>(1)</sup> They also differ in their relative abilities to fix complement, to cross the placenta, and bind to macrophages.<sup>(1,2)</sup> The lack of tryptic peptide differences between these subclasses has made it difficult to make polyclonal antiserum specific to each subclass. However, advent of monoclonal antisera should surmount the problem.<sup>(1,3,4)</sup>

Serum concentrations of IgG subclasses in sera are essential for the diagnosis and management of some immunodeficiencies, monoclonal gammopathy and some allergic diseases.<sup>(1)</sup> However, there is a great range of each IgG subclass serum concentration. Age, race, nutritional status and geographic distribution are the main factors of the variation.<sup>(2,3,5)</sup>

This study was designed to measure IgG subclass serum concentration in Thai adults in order to determine baseline levels for the diagnosis of IgG subclass abnormalities, particularly in Thais.

### Materials and method

Serum samples were obtained randomly from 31 medical students and stored at  $-20^{\circ}\text{C}$  until used. They were considered to be healthy according to the following criteria :

1. No evidence of overt infection within the preceding two weeks.
2. No fever while the serum was being taken.
3. No history of immune disorder including allergic and autoimmune diseases.

Antisera specific for the heavy chains of human IgG1, IgG2, IgG3 and IgG4 prepared by Janssen Biochemica were used. As mentioned in the

product's catalogue, the specificity for human IgG subclasses has been established by radial immunodiffusion using a panel of 40 sera, all typed for Gm, to control the quantification of IgG subclass levels, 20 isolated paraproteins in a concentration of 0.05% and 10 pathological patients' samples.

To determine the levels of the IgG subclasses, the radial immunodiffusion method was carried out using the following procedures :

1. Agar solution was prepared by mixing 2% agarose in Tris buffer (0.05 mol/l Tris, 0.1 mol/l NaCl and 0.1%  $\text{NaN}_3$ , pH 8.0), containing 6% polyethylene glycol (PEG 8000) in a ratio of 1 : 1 v/v.

2. The appropriate amount of each antiserum was added to the 2% agar gel at  $56^{\circ}\text{C}$  (Table 1)

3. The antiserum-agar solution was mixed thoroughly then pipetted onto suitable templates, 1 ml each.

4. The gel was allowed to cool and set.

5. 2 mm-diameter wells were punched into the gel.

6. Standard sera and samples were diluted to the appropriate dilution for each subclass determination (Table 2)

7. 3  $\mu\text{l}$  of each sample and standard sera were dropped into the wells.

8. The plates were left in a moist chamber at room temperature for at least 48 hours.

9. The diameter of precipitation rings appearing on the gel was measured.

10. The computer program "LOTUS 1-2-3" was used to create a linear regression equation of the known standard concentrations and their diameters squared.

11. The ring diameter of each sample was measured and then the subclass concentration was calculated from the linear regression equation.

Table 1. Appropriate amount of IgG subclass-antiserum mixed into 2% agar gel.

Antiserum	Amount of antiserum (ml)	Amount of agar solution (ml)
anti-IgG <sub>1</sub>	0.5	5.5
anti-IgG <sub>2</sub>	0.6	5.4
anti-IgG <sub>3</sub>	0.45	5.55
anti-IgG <sub>4</sub>	0.45	5.55

**Table 2.** Appropriate dilution of standard serum and sample for each subclass determination.

Subclass	Standard concentration	Dilution for standard 1	Dilution for standard 2	Dilution for standard 3	Dilution for sample
IgG1	610 mg/dl	1:2	1:10	1:20	1:20
IgG2	330 mg/dl	1:3	1:6	1:15	1:12
IgG3	47 mg/dl	undiluted	1:2	1:4	1:2
IgG4	62 mg/dl	undiluted	1:2	1:4	1:2

## Results

Thirty-one medical students (18 males and 13 females) were randomly selected. All of them were considered to be healthy according to the inclusion criteria. Because the radial immunodiffusion method is a semi-quantitative measurement. The serum concentration of each subclass was averaged

geometrically. The normal ranges were obtained by taking the mean logarithm  $\pm$  twice the standard deviation of the logarithms and then taking the antilogs of the results. The geometric means of IgG1, IgG2, IgG3 and IgG4 were 1,031.4, 476.2, 48.7 and 78.3 mg/dl, respectively (Table 3). IgG4 levels appeared to be undetermined in two of the serum samples.

**Table 3.** Serum immunoglobulin-G subclasses level of Thai adults.

Subclass	n	Geometric mean	Range
IgG1	31	1,031.4	642-1,655
IgG2	31	476.2	203-1,116
IgG3	31	48.7	24.7-96.2
IgG4	29	78.3	32.8-186.8

## Discussion

Immunoglobulin-G can be divided to four subclasses. IgG1 is the major subclass involved in tissue protection. IgG2 seems critically involved in tissue protection versus carbohydrate antigen and polysaccharides, including bacterial capsules, which tend to be T-cell independent.<sup>(1,6,7)</sup> IgG2 also activates complement thereby mobilizing a cellular response via products of the complement cascade. IgG3 is the major complement activator of the subclasses and participates in the IgG response to protein antigens. IgG4 appears to bind to mast cells, to basophils, and to be involved in immediate hypersensitivity as well

as respiratory tract protection.<sup>(6,8,9,10)</sup> IgG4 usually represents a late antibody response to a high level of antigen.<sup>(9)</sup> Various foods, venoms, inhalant and parasitic allergens can induce IgG4-specific antibodies.<sup>(1,9,10-12)</sup> IgG4 becomes prominent upon chronic antigen stimulation as found in allergen immunotherapy.<sup>(13)</sup> It has been suggested as an allergen-blocking antibody having a protective rather than a pathogenic role.<sup>(9,6)</sup> IgG4 may also have a specific local accumulation in lung fluids.<sup>(6)</sup> Serum IgG subclass abnormalities have been noted particularly in patients with monoclonal gammopathies and immunodeficiencies as well as some other disorders<sup>(14,7)</sup> (Table 4).

**Table 4.** IgG subclass serum concentration abnormalities and diseases.

	IgG1	IgG2	IgG3	IgG4
<b>Infections</b>				
Otitis media in children	—	↓	—	—
Acute respiratory infection	—	↓	—	—
Bronchiectasis	—	—	—	↓
Mother of children with beta-strep	↓	↓	↓	—
Schistomiasis, filariasis	—	—	—	↑
Recurrent sino-pulmonary infections	—	↓	↓	—
<b>Immune disorders</b>				
SLE	↑	↓	—	↓
Juvenile diabetes	—	↓	↓	—
Allergy : Asthma, atopic dermatitis	—	—	—	↑
Multiple sclerosis (CSF)	↑	—	—	—
Hyposensitization therapy	—	—	—	↑
<b>Other</b>				
Advanced melanoma	—	—	—	↑
Burkitts lymphoma	—	↓	—	—
Ataxia telangiectasia	—	↓	↓	↓
Impaired lung function	—	↓	↓	—

Determination of IgG subclass abnormalities is important in epidemiologic studies of both myeloma and immunodeficiencies. Furthermore, the prognosis of diseases may be predicted as some studies showed that patients with low IgG2 levels tend to have greater risk of developing infections, especially respiratory tract infections with the encapsulated bacteria, Haemophilus influenza and Streptococcal pneumoniae.<sup>(1,7)</sup> Individuals with low IgG2 levels are also respond poorly to immunization with carbohydrate antigens.<sup>(1)</sup> Some asthmatic patients with raised serum IgG4 showed poor response to disodium cromoglycated treatment.<sup>(11,10)</sup> However, laboratory interpretation of IgG subclass abnormalities should be done cautiously. Since there are many factors that

bring about variation of serum IgG subclass concentration in normal individuals. Our results reveal some differences compared with the report of Schur PH<sup>(1)</sup> (Table 5), the reasons for which should be attributed to racial and geographic distribution of the subjects. However, it is conceivable that the specificity of the antisera and the laboratory method being used are the other two reasons for the differences.

A few of our samples, like those of other studies,<sup>(1,3)</sup> were found to be undeterminable for IgG4 concentrations. It is possible that the extensive absorption of the polyclonal antisera being used resulted in an IgG4 antiserum which did not detect a subpopulation of IgG4, such as the 4a and 4b subtypes.<sup>(3)</sup>

**Table 5.** Comparison of IgG subclass serum concentration of normal adults.

		IgG1	IgG2	IgG3	IgG4
Our data	mean	1,031.4	476.2	48.7	78.3
	range	642 – 1,655	203 – 1,116	24.7 – 96.2	32.8 – 186.8
Schur PH	mean	540	210	58	60
	range	280 – 1,020	60 – 790	14 – 240	11 – 330

## Summary

Recognition that IgG consists actually of four subclasses has opened up a vast amount of knowledge regarding the clinical correlations of diseases with each IgG subclass. Of particular interest has been the understanding that various antibodies are restricted to some subclasses. The possible role of IgG4 in immunization, allergy and immunotherapy has been determined. IgG subclass deficiencies are being increasingly recognized, especially in association with infection. The normal range of each subclass concentration based on this study can be appropriately used as a guideline for detection of IgG subclass abnormalities, particularly in Thai adults.

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