Quantitative Determination of Immunoglobulins in Cerebrospinal Fluid

Sadao Takase and Motoaki Yoshida

*Division of Clinical Neurology (Prof. K. Itahara), Institute of
Brain Diseases, Tohoku University School of Medicine, Sendai*

A modification of Merrill *et al.*'s method is described for determination of IgG in cerebrospinal fluid in diseases of the central nervous system. The following electrophoretic conditions for 10 μ l of nonconcentrated cerebrospinal fluid were satisfactory: current density of 60 mA, potential difference of 90 V, and electrophoretic time of 90 minutes. Serial dilutions of a standard IgG specimen were used as controls each time together with test samples. In normal subjects the IgG level in cerebrospinal fluid ranged within 1.44 ± 0.53 mg/100 ml. It was higher in multiple sclerosis, postvaccinal encephalitis, measles encephalitis, neurosyphilis, meningitis and obstruction of the spinal canal, but lower in genuine epilepsy. IgG in cerebrospinal fluid in degenerative diseases and myeloneuropathy with abdominal signs (SMON) were within normal range. In brain tumors no constant IgG pattern was found.

IgG% (IgG concentration/total protein concentration $\times$ 100) in multiple sclerosis, postvaccinal encephalitis, measles encephalitis, tuberculous meningitis and neurosyphilis was increased, but that in obstruction of the spinal canal remained within the normal range.

It is well established that several kinds of serum immunoglobulin are identified by the immunoelectrophoretic technique.^{1,2} But when this method is applied for cerebrospinal fluid (CSF), a large amount of concentrated CSF is required.

Recently, Merrill *et al.*³ described in detail an electro-immuno-diffusion (EID) method for the quantitative determination of immunoglobulins in cerebrospinal fluid. According to them, the length of the antigen-antibody precipitate band is proportional to the concentration of antigen in the well and to the duration of electrophoresis. This method requires a considerable amount of anti-serum.

The purpose of the present paper is to describe a modified method for the determination of IgG and IgA in CSF and their levels in various diseases of the central nervous system (CNS).

METHOD AND MATERIALS

1) *Antiserum.* Goat and rabbit anti-serums to human IgG and IgA, and horse anti-serum to whole human serum were supplied from Hyland Lab., Los Angeles (Lot No. S101C1) and stored at 4°C until use. The anti-IgG and anti-IgA sera gave a single precipitation line in immunoelectrophoresis using normal human serum as antigen.

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The standard serum for each immunoglobulin was supplied also from Hyland Lab. (Lot No. 7030D005A1), and from this serum a series of twofold dilutions in physiological saline was prepared for reference. These solutions contained 68, 34, 17, 8.5, 4.25, 2.13, 1.06, 0.53 and 0.26 mg of IgG per 100 ml.

2) *Material.* CSF was obtained by lumbar puncture from patients without any neurological signs or with various neurological diseases. Several samples of CSF were assayed immediately, but most of them were stored at -20°C after centrifugation to remove leukocytes.

3) *Experimental procedure.* Total CSF protein was measured by the sulfosalicylic acid method with a photocolormeter at 640 m μ .⁴

Twenty ml of warmed 1% Difco's Noble agar sol in veronal buffer (pH 8.6, $\mu=0.05$), to which 2.0, 0.4, 0.2 and 0.1 ml of anti-IgG serum had been added, were overlaid on four pieces of slide glass and gelatinized at room temperature. Three small circular wells were punched on a line along the short axis of each slide, and the agar in the wells was removed by gentle suction. Then, 4, 8 and 10 μl of reference solution of IgG and CSF were placed in each well and were examined at room temperature under the following electrophoretic conditions: current density of 5–20 mA per each slide, potential difference of 50–150 V and electrophoresis time of 60–180 minutes. The electrode chambers contained pH 8.4, $\mu=0.05$ veronal buffer. After electrophoresis the slides were washed in saline for 12–24 hours in a chamber at 37°C , in distilled water for 1–12 hours, and then dried and stained with 0.5% amido-black 10-B solution for 15 minutes.⁵

The length of each standard precipitation band was plotted on the abscissa and the immunoglobulin concentration on the ordinate of a semi-logarithmic scale, and the concentration of IgG in test samples was determined from the reference curve.

4) *Clinical cases.* Experimental samples were obtained mainly from patients with various diseases of the nervous system and myopathy hospitalized in the Division of Clinical Neurology and Neurosurgery, Institute of Brain Diseases, Tohoku University School of Medicine, Sendai.

RESULTS

The pattern of a precipitate on the slide toward the cathodal direction is shown in Fig. 1, and the relation between the concentration of anti-serum to human IgG and the length of the precipitate line in Fig. 2.

The curve of (a) in Fig. 2 is slightly concave upwards, but (b) and (c) show straight lines. It is clear that (b) and (c) lines are suitable for quantitation of IgG,

Fig. 1.

Fig. 2. Relation between concentration of antiserum and the length of precipitate.

(a) $50\times$, (b) $100\times$ and (c) $200\times$ concentration of antiserum in agar plate.

Electrophoretic condition: 60 mA, 100 V, 90 min.

and furthermore, the form of precipitate had a more sharply defined contour in (b) than in (c). The reference curves of IgG solutions containing 8.5, 4.25, 2.13, 1.06, 0.53 and 0.27 mg per 100 ml exhibited a clear linearity under the following conditions: current density of 60 mA, potential difference of 90 V, electrophoretic time of 90 minutes and volumes of $10\mu\text{l}$ in each well (Fig. 3). When the duration of electrophoresis was within 120 minutes, the relationship between IgG concentration and precipitate was linear as seen in Fig. 4, while in the longer duration the

Fig. 3. Correlation between IgG concentration and precipitate length.

Fig. 4. Various duration times of electrophoresis.

Duration times of electrophoresis: (a) 60 min, (b) 90 min and (c) 120 min.

linear relationship was disturbed. As for electrophoretic potential, the above linear relationship was obtained except for the range of 90–100 V.

The sample volume in a well and its relation to the length of precipitate line were studied as shown in Fig. 5, and it was found that 10 μ l (b) were more suitable than 4 μ l (a), because in the latter the form of precipitate could not be clearly demonstrated in lower antigen concentrations. The length of precipitate line was

Fig. 5. Correlation between each sample in a well and the length of precipitate.

dependent upon the volume and concentration of the sample in a well. For this reason, the length of precipitate band was decided by the sample concentration when the sample volume was constant.

The electrophoretic conditions satisfactory for the experiment were as follows: current density of 60 mA (15 mA in each slide), potential difference of 90 V, electrophoretic duration of 90 min and the sample volume of 8 or 10 μ l in a well.

There is a problem of the reproducibility of experimental results from the reference materials. We found a close agreement between two reference curves obtained from the same agar plate, that is, under the same electrophoretic and other conditions (Fig. 5, (b)). The same volumes of serial dilutions of the reference

Fig. 6. Reproducibility of EID to the standard subjects.

material of IgG were examined five times under the same electrophoretic conditions (60 mA, 90 V and 90 min), and the concentrations were plotted on a semilogarithmic scale against the length of precipitate lines as shown in Fig. 6. Close agreement among five separate experiments was not always found, but the curves were nearly straight and parallel to one another. As expected, the variation in temperature during electrophoresis and other conditions at the time of preparing agar plates influenced the length of precipitate lines. Each straight line is expressed as $Y=aX$, where Y is IgG concentration, a is a constant and X is the length of precipitate band. Accordingly, when serial standard solutions of IgG are used as controls on the same slide in each electrophoresis, IgG concentration of test samples can be easily detected.

IgG concentration in various diseases of CNS

The mean and standard deviation of immunoglobulin values in CSF of 21 normal subjects were 1.44 ± 0.53 mg/100 ml, extreme values ranging from 0.5 to 2.5, as shown in Table 1. The levels of IgG (mg/100 ml), IgG % which was expressed as IgG per cent of total protein content in CSF, and the total protein levels in CSF were shown in Table 2 and Fig. 7.

TABLE 1. *Mean levels of immunoglobulin in normal CSF*

Authors	IgG (mg/100ml)	IgA (mg/100ml)
Merill <i>et al.</i>	1.0~3.1	
Iwashita & Hayashi ⁷	4.0 (2.5~6.3)	
Gottesleben & Bauer ⁸	1.76 ± 0.412	0.226 ± 0.19
Itoh <i>et al.</i> ⁸	2.1 (1.0~3.5)	
Takase <i>et al.</i>	1.44 ± 0.53 (0.5~2.5)	0.2 (0.1~0.3)

TABLE 2. *Mean levels of CSF immunoglobulins in various diseases*

Diagnosis	Number of cases for examination	Total Protein in CSF (mg/100 ml)	IgG Concentration of CSF (mg/100 ml)	IgG % of CSF IgG conc./total protein $\times 100$	IgA Concentration of CSF (mg/100 ml)
Normal subjects	21	31.5	1.44 ± 0.53 6.3	4.44 ± 1.25	0.2
Multiple sclerosis	3	55	(4.0~9.5) 4.9	10.6	1.14
Postvaccinal encephalitis	5	48	(3.2~9.0) 6.0	10.2	0.54
Measles encephalitis	3	77	(3.9~8.2) 2.3	7.8	1.03
SMON	12	33	(1.5~4.2) 2.1	6.9	0.42
Motor neuron diseases	7	39	(1.0~4.2) 5.7	5.4	0.2
Obstruction of spinal canal	8	103	(1.8~16.0)	5.5	1.16
Atrophy of the brain of unknown origin	6	37.4	1.62 (0.8~2.6)	4.3	0.2
Neurosyphilis	4	66	14.0 (6.2~28.0)	21.2	3.2
Meningitis tuberculosa	3	75	10.8 (4.2~19.0)	14.4	2.7
Genuine epilepsy	10	29	0.74 (0.1~2.8)	2.6	
Cerebral infarction	7	43	2.17 (1.1~4.8)	5.1	0.4
Brain tumors	14	51	2.7 (0.3~8.5)	5.3	
Myasthenia gravis	3	45.3	2.7 (1.0~5.1)	5.96

Fig. 7. IgG concentrations in cerebrospinal fluid of various diseases.

The total protein levels and IgG levels in CSF were higher in multiple sclerosis, postvaccinal encephalitis, and obstruction of the spinal canal, especially in neurosyphilis and tuberculous meningitis.

In several cases of genuine epilepsy, the total protein in CSF was not so low, while the IgG level and IgG% showed low values.

IgG levels in CSF were within normal range in degenerative diseases of the brain and the spinal cord comprising amyotrophic lateral sclerosis, diffuse atrophy of cerebellum, and subacute myelo-optico-neuropathy with abdominal sign (called SMON informally in Japan).

IgG levels in CSF in brain tumors did not show any constant pattern, in which increased IgG was found in cases of tumors of abundant vascularization. Under long-term steroid treatment decrease of IgG in CSF was observed.

IgG % increased markedly in multiple sclerosis, postvaccinal encephalitis, tuberculous meningitis and neurosyphilis, and increased moderately in measles encephalitis and SMON, while in obstruction of the spinal canal it was within normal range in spite of marked increase of IgG.

DISCUSSION

Electro-immunodiffusion method (EID) for CSF was reported recently by Merrill *et al.*,³ and this method is said to be a rapid and sensitive one for the determination of immunoglobulins in a very small amount of dilute or un-concentrated biological fluids. According to their report,³ the length of antigen-antibody precipitate line is dependent upon the concentration of IgG in the

solution and not upon the amount of IgG in the well. However, our experiment revealed that the sample volume and the antigen (IgG) concentration influenced the precipitate length by EID, as shown in Figs. 2, 3 and 5.

On the problem of reproducibility of EID using standard specimen, we recognized almost complete agreement between two standard curves obtained from the same agar plate (Fig. 5, (b)). But, even when the same volumes of serial concentration of the standard serum were used under the same electrophoretical conditions in several experiments, we did not always find such a close agreement. It is clear that obtained straight lines are parallel as seen in Fig. 6. Therefore, when standard series of IgG is used on the same slide for each electrophoresis, IgG concentration of test samples can be easily detected from the reference curve.

IgG in CSF from 21 normal subjects determined by this method ranged from 0.5 to 2.5 mg/100 ml and averaged 1.44 ± 0.53 mg/100 ml as shown in Table 1 and Fig. 7, and the total protein content of their CSF was lower than 45 mg/100 ml. IgG values in normal CSF vary according to authors,⁶⁻⁸ but this variation may be reduced in future by unification of experimental procedures. It has been known that γ -globulin fractions of CSF increase in chronic and subacute infectious diseases of the central nervous system, particularly neurosyphilis and tuberculous meningitis, multiple sclerosis, subacute sclerosing leuco-encephalitis and other neurological diseases.^{9,10}

In multiple sclerosis, postvaccinal encephalitis, measles encephalitis, tuberculous meningitis and particularly incomplete obstruction of the spinal canal, both the total protein and IgG levels in CSF were found to be markedly high in our experiment. Increase of IgG, however, could not be concluded unless the possibility of protein concentration by obstruction is excluded.

Next, IgG % expressed as percentage of IgG concentration to total protein concentration in CSF was extremely high in multiple sclerosis, postvaccinal encephalitis, neurosyphilis and tuberculous meningitis, and moderately high in measles encephalitis and SMON, while it remained within normal range in cases of incomplete obstruction of the spinal canal. IgG % in degenerative diseases of CNS including mainly cerebral and cerebellar atrophy, amyotrophic lateral sclerosis and brain tumors was equal to or a little higher than that of normal subjects.

In most cases of genuine epilepsy, in which the total protein was not so decreased in spite of decrease in IgG concentration, IgG % was slightly decreased, but that in symptomatic epilepsy did not show a constant pattern. The γ -globulin concentration in epileptic convulsion was nearly equal to that of normal subjects in Goodman's paper.¹¹

On the problem of IgG production in CSF, there is a view of local biosynthesis of IgG in the central nervous system.

Firstly, from the present results showing that a high value of IgG and a normal level of IgG % was found in cases of incomplete obstruction of the spinal canal, it is clear that CSF protein was concentrated on account of disturbed circulation of CSF, because IgG% remained within normal range.

Secondly, extremely high values of both IgG and IgG % in neurosyphilis and tuberculous meningitis may be explained by the assumption that the permeability of the blood-brain barrier is increased and the production of IgG is accelerated in the region of inflammation.

Thirdly, IgG and IgG % in CSF were high in multiple sclerosis and moderately high in SMON and measles encephalitis. If immunoglobulin would be exclusively derived from the plasma into the CSF, it would be difficult to explain how serum IgG in these diseases usually remains normal (Fig. 8).

Fig. 8. IgG Concentrations in serum of various diseases.

The discrete γ -subfractions in CSF are considered to be the products of plasma-cell clones which proliferate in the local lesions of the central nervous system as a response to antigen of exogenous or endogenous origin. So, our experimental results seem to support the opinion of local biosynthesis of IgG.

The determination of immunoglobulin levels in CSF in various diseases seems to contribute much to diagnosis and prognosis of various diseases of the central nervous system.

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