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Utility of Turbidimetric Procedure for Assaying Immunoglobulins in CSF: A Preliminary Report

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Abstract

The turbidimetric procedure for quantitative estimation of immunoglobins in serum is modified for CSF, Serum and CSF levels of IgG and IgA are measured in normals.

Key words -
CSF,
Serum,
Immunoglobins,
Turbidimetric method

Since Heidelberger and Kendall [1] introduced a quantitative immunoprecipitation technique, many methods have been developed in the field of immunoglobins. The development of immuno-diffusion and double diffusion techniques have increased the utility of the quantitation and identification of the antigens present in body fluids. Various methods for quantitation of antigens in body fluids have been developed and applied for diagnosing conditions associated with immunoglobulinopathies.

Immunochemical techniques such as radial immunodiffusion [2] nephelometry [3], immuno electrophoresis [4], electroimmunodiffusion [5] and immunonephelometry [6] are some of the currently available methods for accurate quantitation of immunoglobulins as well as proteins of clinical importance

These techniques are simple enough to be used in any laboratory. However, when specificity and sensitivity are needed, especially in case of low protein containing body fluids, such as CSF, some of these procedures have limitations. Since the detection limit of immunoglobulins by these procedures are well above the normal levels of immunoglobulins in CSF, these procedures cannot be used for analysing CSF immunoglobulins. Recent advances in immunological disorders have shown the importance of CSF immunoglobins as diagnostic aids in many neurological disorders such as multiple sclerosis. The only method which reaches the sensitivity for detection of immunoglobulins, like lgG in CSF, is the quantitative nephelometric method. But this cannot be directly used to analyse the low level immunoglobulins such as IgM and IgA. In order to assay these immunoglobulins in CSF of patients with various neurological disorders, this

procedure was modified slightly and employed. The details are given in this paper.

Material and Methods

CSF collected from 30 patients without neurological or neurosurgical problems but who were operated for systemic disease served as control samples. Simultaneously a blood sample was collected and serum separated. Serum immunoglobulins (IgG, IgM and IgA) were analysed by the kinetic measurement of antigen/antibody reaction by the fixed-time procedure of Neumann [7]. CSF immunoglobulins were assayed by a modification of the same method, details of which are as follows. The reagents and standards were obtained from Ms. Boehrninger Company, Mannheim (West Germany). The serum samples were diluted 1+10 with normal saline. An aliquot of diluted serum (10µ l for IgG, 50µ l for IgA and 100µ l for IgM) was mixed with 1.0 ml of buffered antiserum specific for each immunoglobulin. The resulting turbidity was measured at 340 nm at 25° C after exactly one and five minutes. For this purpose a spectrophotometer with programmable printer giving OD upto fourth decimal place was used (Gilford Stasar III System 4). Immunoglobulin standards in the range of 90-500 mg% of IgA, 60-3000 mg% of IgM and 500-300 mg% of IgG were processed and assayed similarly. The measured difference in absorbance is plotted against the specific concentration on linear graph. The immunoglobulin concentrations in the samples are obtained from the curve by reading against the measured absorbance difference. For CSF a slight modification of procedure was done. A separate standard graph was plotted by using immunoglobulin standards in the range of 10-50 mg% of IgA and IgM and 50-300 mg% of IgG. The standards were diluted 1+10 with normal saline. CSF was used for analysis without dilution. Both standards and samples were processed and assayed similar to serum analysis. The delta absorbance difference for 5 minutes was used for calculating the immunoglobulin concentrations from the standard graph (Figs 1 & 2). The values obtained from the graph were divided by 11 (dilution factor) to get the immunoglobulin concentration in CSF. With the use of four digit readout in the Stasar instrument it was possible to detect even the slightest change in OD.

- ***Standard graph for IgM & IgA***
- ***Standard graph for IgG***

Results

The serum and CSF immunoglobulin levels in controls are indicated in Table I. As could be seen from this Table, the serum IgG levels range from 890 to 1010 mg% with a mean of 960.0 ± 66 and IgM levels range from 120.0 to 155 mg% with a mean of 128.0 ± 17.4. The IgA levels were in the range of 140 to 170 mg% with a mean level of 156 ± 10.5.

Table 1 - CSF and Serum Immunoglobulin in Normals .
mean values with SD

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Table II - Comparison of CSF & Serum Immunoglobulin (Mean Values) by Different Methods

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The CSF IgG levels were in the range of 1.8 to 3.5 mg% (2.25 ± 0.5) whereas IgM levels were in the range of 0.15 to 0.20 mg% (0.19 ± 0.03). Almost all CSFs showed the presence of IgA. The levels were in the range of 0.02 to 0.04 mg% (0.02 ± 0.01).

Discussion

Various immunochemical methods have been used for estimation of immunoglobulins in CSF. But each one has its limitation for CSF since the levels of IgA and IgM and very low in CSF which makes it difficult to detect by these methods.

The immunoprecipitation method described by Kabat [8], when applied for CSF detected only IgG and IgM. The electroimmunodiffusion method of Merill et al [9] showed that IgG constitutes nearly 1/16th of total CSF protein. Here also the levels of IgA and IgM were not detectable in normal CSF. Toutellote et al [10] have applied this electroimmunodiffusion test in the diagnosis of multiple sclerosis, and have reported that the normal CSF IgG level ranges from 0.9 to 8 mg% with a mean of 2.7. Single radial immunodiffusion method of Mancini was found to be accurate for IgG but lacked sensitivity for IgA and IgM levels (Table II). Some procedures required concentration of CSF proteins prior to application. This has the inherent danger of denaturation unless carefully carried out. The nephelometric procedure is found to be more sensitive for IgG and IgM. By the modification it was possible to detect IgA levels also in CSF. Since the involvement of CSF immunoglobulins in some of the neurological disorders such as in multiple sclerosis, ataxia telaangiectasia, optic neuritis, among others, has been well established, the assay of these immunoglobulins has gained much prominence. For this, more sensitive, specific and quick methods are necessary. The turbidimetric method satisfies these criteria. This procedure is being used as a diagnostic tool in certain neurological disorders at this centre.

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