Comparison of Electroimmunodiffusion and Radial Immunodiffusion for Measurement of IgG in the Laboratory Diagnosis of Multiple Sclerosis

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The detection of a supranormal quantity of immunoglobulin G (IgG) in cerebrospinal fluid is valuable in diagnosis of multiple sclerosis. In a previously published study of over 300 patients, including 46 patients with a clinical diagnosis of multiple sclerosis, 74% of the patients were found to have an abnormally high IgG value, expressed as a ratio of IgG.albumin, by the reliable method of electroimmunodiffusion. In the present study the recently introduced method of radial immunodiffusion was compared with established electroimmunodiffusion technique in divided samples of cerebrospinal fluid from 49 patients with a wide range of IgGalbumin values. The correlation of the results obtained by these two methods was very good (r = 0.945), but the variance with radial immunodiffusion (±20%) is greater than with electroimmunodiffusion (±15%). Two samples that exhibited increased values by the electroimmunodiffusion method were normal with the other technique. We conclude that electroimmunodiffusion remains a slightly more accurate and sensitive technique, although radial immunodiffusion is simpler and requires less technician time.

Additional Keyphrases: "kit" method • IgG/albumin ratio in CSF • normal values

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The search for a relatively simple laboratory test for multiple sclerosis began with the observation by Kabat et al. (1) that the ratio of gamma globulin to total protein in CSF² was increased in many patients with this disease. So expressed, the concentration of gamma globulin was increased in about 75% of patients with multiple sclerosis, even though the absolute concentration of gamma globulin alone in CSF was not always increased. By quantitative precipitation of the CSF gamma globulin with anti-human gamma globulin and use of one of several methods to determine CSF total protein, the ratio was measured (2). The original technique required from 0.5 to 2.0 ml of CSF, a considerable amount of tedious work, and was neither simple nor rapid enough for routine use in clinical laboratories.

After this method had been in use in large centers for many years, the process of EID was developed and applied to measuring IgG in CSF (3). Later, Tourtellotte refined the method of EID to measure both IgG and albumin simultaneously in a single drop of CSF (4). He demonstrated that the total protein concentration correlated very closely with the albumin concentration, and therefore that the gamma globulin in CSF could be expressed in terms of a ratio

² Nonstandard abbreviations used: CSF, cerebrospinal fluid; EID, electroimmunodiffusion; RID, radial immunodiffusion; and Ig, immunoglobulin.
of IgG to albumin instead of IgG to total protein (5). Details of special technical problems with the EID technique have been elaborated previously (4, 6).

We have analyzed 302 samples of CSF by Tourtelotte's technique and have compared the values to published data for the IgG/albumin ratio in CSF (6). Our results correspond closely to other published data of this kind for multiple sclerosis (5, 7), and show that about 75% of patients with an established clinical diagnosis of multiple sclerosis had increased IgG values for their CSF.

Recently Berner et al. (8) described a two-step method to arrive at the IgG/total protein ratio for CSF. This involved the RID technique for estimating the concentration of IgG and the routine chemical determination of total protein, and is in many ways simpler and more adaptable for most clinical laboratories than EID. Hyland Division of Travenol Laboratories has now developed a test in which both IgG and albumin in CSF are measured by RID. This paper reports the IgG/albumin ratios for 49 samples of CSF, as measured by both the EID and the commercially available methods.

Methods

EID was done by Tourtelotte's method (4). A gel plate was prepared with equal parts of agarose ("SeaKem" Marine Colloids, Inc.) and Special Agar Noble (DIFCO) in diethylbarbiturate buffer (pH 8.6, ionic strength 0.05) at a final concentration of 1 g/dl, to which antisera to human albumin and IgG were added. High titer antisera, prepared by us, were used because the dilute nature of commercially available antisera adds to the technical difficulty of the test as well as greatly increasing the cost. A "Kimax" micropipet was used to quantitatively pipet 5 µl of CSF into wells cut in the hardened gel. The electrophoresis power was "on" during the pipetting and the power remained "on" for 2.5 h (28 mA, and 60 V) (CAUTION). Incidentally, results varied with the type of electrophoretic chamber used. Therefore, a Thomas Electrophoretic Cabinet, Model 20, was used for all determinations. The lengths of the rocket-shaped precipitate arcs were measured to the nearest 0.1 mm immediately upon completion of the test and their respective values were calculated from a graph of the standards on log-log paper.

RID was done with Hyland's IgG–albumin "immunoplates," lot No. 7132T001131, according to the method recommended for the procedure. These immunoplates are designed so that two separate sections of agar contain either antihuman IgG or antihuman albumin. Each sample of CSF was placed in a well in each section. At least four known standard solutions were used in each section if only one plate was used. If several plates were used, four standards were placed in only one plate and one standard was used in all other plates. The plates were incubated for 4 h at 37 °C, then soaked in dilute acetic acid for 1 min. The diameters of the precipitate rings were then measured to the nearest 0.1 mm and the values for albumin and IgG were calculated from a graph of the standards on semi-log paper (diameter on the linear axis, concentration on the logarithmic axis). For those plates with only one standard, the standard curve was adjusted, if necessary, to match that standard for best results.

Initially, 302 CSF specimens were collected from patients ranging in age from 2 months to over 70 years, and the EID method was used for samples that either were fresh or had been stored frozen without being thawed more than twice. A group of 49 specimens was evaluated by both methods simultaneously. These 49 samples were selected because the group as a whole exhibited a wide range of values for the IgG/albumin ratio, as measured by the EID method. We paid no attention to the clinical diagnosis. The two values were paired and the correlation coefficient was determined by a standard formula (9).

Results

Comparison of results obtained by the two methods showed that they correlated with each other very well (correlation coefficient, r = 0.945). Two samples of the 49 involved in the comparison had an elevated ratio by one method (EID) and a normal ratio by the other (RID). Values from each method were about the same. This was verified by plotting the paired data with the two values on different axes and determining slope and intercept of the best-squares linear-regression line (Figure 1). The slope was 1.14 (48 °) and the ordinate intercept −2.7 when the RID scale was plotted on the ordinate and the EID scale on the abscissa. A paired t-test of the data yielded a value of 0.65, which supports the other statistical observations. However, the variance for the RID method (±20%) was slightly more than that for the EID technique (±15%).

Fig. 1. Paired values of CSF IgG/albumin concentrations, expressed as percentages, as determined by two methods, electroimmunodiffusion (EID) and radial immunodiffusion (RID)

The two methods correlate closely. The blocked-off area (lower left) represents the normal zone and the line is the regression of the values shown.
Discussion

The values of the IgG/albumin ratio for CSF correlate quite closely when one compares the EID and RID techniques. However, we found somewhat greater variation with the RID than with the EID method, not surprising when one considers the possible sources of error with the two measurements. First, with the EID method 5 μl is added quantitatively to the well, whereas the RID method simply calls for filling the well to the brim. Second, one might expect that this potential filling error would be compounded by the use of two wells in the RID system and only a single well with the EID method. EID also has a theoretical advantage in that the antigens migrate in a single direction, giving a greater distance to be measured in the quantitative process. One must concede that EID is probably a more accurate and sensitive method for antigens in lower concentrations (10).

We concluded that the RID method for the measurement of CSF IgG and albumin correlates quite well with the EID technique, but that the former has more inherent variation than the latter. EID remains a more accurate and sensitive technique, despite the fact that RID is simpler and requires less laboratory technician time.

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