COMPARISON OF VIRAL RNA ELECTROPHORESIS AND INDIRECT ELISA METHODS IN THE DIAGNOSIS OF HUMAN ROTAVIRUS INFECTION

Luis F. Avendaño, Sylvia Dubinovsky, and Harvey D. James, Jr.

A relatively cheap and easily applied method for detecting rotavirus pathogens (viral RNA electrophoresis) was tested against the indirect ELISA method, using stool samples from 177 Chilean infants and young children. This article presents the results of that comparative research.

Introduction

It has been estimated that roughly 420,000 children under five years of age die annually in Latin America, most of the victims being less than a year old. Diarrheal disease syndromes are believed responsible for roughly one-fifth of these deaths (1). The numbers of actual diarrheal disease cases reported have been relatively low (on the order of 250,000 cases per year) because of statistical underregistration in some countries. Nevertheless, even these figures give some idea of this health problem’s importance (2).

In Chile, it has proved possible to isolate the probable causative agents (bacteria, parasites, or viruses) in approximately 60% of the infant diarrhea cases subjected to investigation. Nevertheless, even when modern laboratory techniques are applied, in a significant share of the cases the etiology remains uncertain (3). This circumstance directs attention to the importance of the techniques for diagnosing rotavirus diarrhea. These viruses have been increasingly recognized as important diarrheal disease agents, especially since electron microscopy detected them in stool samples from children with diarrheal disease syndromes in 1973 (4). These and other findings have since shown a clear and positive correlation between the presence of rotavirus and diarrhea in anywhere from 30% to 80% of the diarrhea cases studied, the percentages varying with the conditions of the particular study involved (4-7). Overall, the combined result of this research has been to single out rotavirus as the etiologic agent most frequently involved in the pathogenesis of communicable infant diarrhea.

Rotavirus diarrhea can be diagnosed with high precision by electron microscopy. However, the cost of the necessary equipment and associated technology limits the potential for applying this method routinely in daily practice. Therefore, so as to permit detection and study of these viruses in the ordinary laboratories of health or research facilities, alternative procedures have been developed (8,9).

One procedure, viral RNA electrophoresis in agarose gels, developed by Espejo and colleagues (10), is based on the fact that rotavirus nucleic acid can be identified electrophoretically because it consists of double-stranded RNA divided into 11 segments which have characteristic migration patterns. The procedure can be used to identify both rotaviruses and para-rotaviruses. All in all it is a cheap, simple, and highly specific
diagnostic method; however, its sensitivity has not yet been determined.

Another procedure, the enzyme-linked immunosorbent assay (ELISA), based on antibody-antigen reactions, appears to offer a method similar to radioimmunoassay that is cheap, simple, quick, and highly sensitive. This method, accepted as a rotavirus diagnostic procedure by scientific centers throughout the Americas, is now in widespread use (8, 9, 11). However, the method does involve certain problems—such as the occurrence of false positive results and difficulties experienced by laboratory personnel in preparing the necessary reagents on their own. The problem of nonspecific positive results is especially pronounced with regard to available commercial tests utilizing the direct ELISA method (12), because these do not include controls intended to reduce nonspecific positive readings, and so this method’s use in clinical or scientific research is not universally accepted. In contrast, the indirect ELISA method (used by the United States National Institutes of Health) tests specimens with both preimmune and postimmune (hyperimmune) goat sera in order to reduce the problem to a minimum. The object of the work reported here was to compare results obtained with viral RNA electrophoresis in agarose gels to those obtained with the indirect ELISA method in order to assess the former method’s capacity to detect rotavirus in stool samples.

Materials and Methods

Detection of rotavirus by the two techniques was compared partly by using each to test 50 stool samples obtained at random from 50 ambulatory and hospitalized children under two years of age with acute diarrhea syndromes. The patients had been admitted to the Infant Unit of the Roberto del Rio Hospital or were seen at outpatient clinics in the Northern Health Area of Santiago. In addition, the two techniques were also tested on 127 stool samples from 42 hospital inpatients of the same age group with protracted diarrheal syndromes (13). Later, when the results were assessed, these 127 specimens were considered in two ways—both as individual specimens (irrespective of the patient they came from) and as serial specimens from 47 patients.

Viral RNA electrophoresis in agarose gels was performed in accordance with the description provided by Espejo et al. (10). Specimens which exhibited the first four of the nine characteristic rotavirus RNA segment migration bands were considered positive.

The indirect ELISA testing was performed at the U.S. National Institutes of Health (8, 11), using the pre-post sera system in which samples were tested with preimmune goat serum and with goat serum hyperimmune to human rotavirus. The results, read by photocolorimetry, were considered positive if the reading obtained with the hyperimmune serum was at least twice as great as that obtained with the preimmune serum. Samples yielding highly nonspecific results were diluted ten-fold and retested.

Results

Of the 50 stool samples obtained from children with acute diarrhea, 18 (36%) tested positively for rotavirus by the electrophoretic method and 23 (46%) yielded positive results by the indirect ELISA method (Table 1). All the samples yielding negative results by the ELISA method also yielded negative results by electrophoresis; but five samples gave negative electrophoretic and positive ELISA results. The net difference between the two sets of results, considering all the samples tested, was therefore 10%. If the ELISA results are used as a standard, then the electrophoretic method appeared to produce results that were 78.3% as sensitive and
Table 2. Results obtained with 127 stool samples from 42 patients with protracted diarrhea. Each sample's response (positive or negative for rotavirus) to the viral RNA electrophoresis and indirect ELISA tests was as shown.

<table>
<thead>
<tr>
<th>ELISA (+)</th>
<th>ELISA (-)</th>
<th>Total</th>
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<tbody>
<tr>
<td>v RNA EP (+)</td>
<td>29</td>
<td>3</td>
</tr>
<tr>
<td>v RNA EP (-)</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>Total</td>
<td>48 (38%)</td>
<td>79 (62%)</td>
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$X^2 = 10.2; 0.01 > p > 0.001.$

100% as specific as the ELISA method.

The results obtained with the stool samples from patients with protracted diarrhea yielded fairly similar results (Table 2). That is, 32 (25.1%) of the 127 samples tested positively for rotavirus by the electrophoretic method and 48 (37.7%) yielded positive results with the indirect ELISA method, the difference between the level of positive results being 12.6%. Three samples yielding negative results by the ELISA method gave positive results by electrophoresis, and 19 samples yielding negative results by electrophoresis yielded positive ELISA results. The indicated sensitivity of the electrophoretic method was therefore 62.7%, while that of the ELISA method was 94.1%.

If these results with samples from patients with protracted cases are assessed in terms of individual patients (all of whom provided at least two serial specimens), it turns out that 12 (28.5%) of the 42 patients were positive for rotavirus by the electrophoretic method and 17 (40.4%) were positive by the ELISA method, the difference between the level of positive results being 11.9% (Table 3). Two patients yielding negative results by the ELISA method gave positive results by electrophoresis, and seven patients yielding negative results by electrophoresis yielded positive ELISA results. The indicated sensitivity of the electrophoretic method was thus 63.2%, while that of the ELISA method was 89.5%.

Table 3. Results obtained with 127 stool samples from 42 protracted diarrhea cases in terms of patient diagnoses. The numbers of patients found by each of the two test methods to have rotavirus in their stools were as shown.

<table>
<thead>
<tr>
<th>ELISA (+)</th>
<th>ELISA (-)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>v RNA EP (+)</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>v RNA EP (-)</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>17 (40%)</td>
<td>25 (60%)</td>
</tr>
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$X^2 = 1.7; .20 > p > .10.$

Discussion

In this study it was observed that the indirect ELISA method detected approximately 10% more rotavirus infections than were detected by viral RNA electrophoresis. This difference could arise from the fact that the electrophoretic method used depends on virus shedding (around $10^7$ to $10^{10}$ virus particles per gram of stool) in order to yield a positive result (10). In contrast, the ELISA method requires a lesser concentration of rotavirus, and it seems likely that the specific antibody involved would react not only with complete viral particles but also with rotavirus fragments or components. These circumstances, together, could account for the greater sensitivity of the latter method (9).

Analysis of the data in Tables 1-3 demonstrates that the electrophoretic method was less sensitive than the ELISA method. We did not calculate the positive predictive value involved, because one of the important features of the electrophoretic method used is its high specificity, which tends to preclude false positive results.

In Chile, utilization of this viral nucleic acid electrophoresis technique has permitted the detection of rotavirus in about 30% of the hospitalized infants with acute diarrhea who have been tested, throughout the year, while yielding no positive results with control specimens (15,16). These findings have demonstrated that rotavirus is one of the most important etiologic agents involved in the genesis of diarrheal disease in our country. Moreover, even though our results have affirmed that this technique is less sensitive than the indirect ELISA method, its high specificity,
ease of application, and low cost compared to the ELISA method (for which reagents are difficult to obtain at present) makes the electrophoretic technique a very useful alternative method that can be employed in clinical and epidemiologic studies of rotavirus infection. In addition, the use of a modified viral RNA electrophoretic technique can provide further information about the electrophoretic pattern of the rotavirus genome, making it possible to establish clinical and epidemiologic correlations with the human rotavirus variants that have been described (17, 18).

Finally, the use of highly sensitive diagnostic techniques, such as the ELISA method employed in this study, has helped to underscore the important pathogenic role played by the rotavirus agent in infant diarrheas, and has thereby shown the need to have relatively simple alternative diagnostic techniques on hand that can be incorporated into the routines of medical center laboratories in developing countries. Therefore, because of these considerations, despite the viral RNA electrophoresis technique’s lesser sensitivity, we believe that it constitutes a useful tool for the study of rotavirus infections.

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SUMMARY

A total of 177 stool samples from Chilean diarrhea patients under two years of age were tested for rotavirus by two methods—the indirect enzyme-linked immunosorbent assay (indirect ELISA) and viral RNA electrophoresis in agarose gels (v RNA EPH). Fifty of the specimens came from patients with acute diarrhea and 127 came from patients with protracted diarrhea. The indirect ELISA testing was performed at the National Institutes of Health in the United States; the electrophoretic testing was carried out in Santiago, Chile by the authors.

The electrophoretic method detected rotavirus in 36% of the acute samples and 25% of the samples from protracted cases, while the indirect ELISA method detected rotavirus in higher percentages of samples—46% and 38%, respectively. These results support the conclusion that v RNA EPH is a less sensitive method for detecting rotavirus than the indirect ELISA. Nevertheless, the former method’s high specificity, ease of application, and low cost make it a worthwhile alternative to indirect ELISA. Thus, considering the important role played by rotavirus in infant diarrhea and the need for a diagnostic technique that can be incorporated into the routines of medical center laboratories in developing countries, there is good reason to conclude that v RNA EPH is a useful tool for studying rotavirus diarrhea.

REFERENCES

(3) Duffau, G. Síndrome diarréico agudo del lac-


