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SHORT COMMUNICATION

RAPID DETECTION OF ROTAVIRUS IN FAECES BY A SLIDE LATEX AGGLUTINATION TEST AS COMPARED WITH AN ENZYME-LINKED IMMUNOSORBENT ASSAY

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Abstract

A commercial latex agglutination slide test for rotavirus detection (Rotascreen^R) was compared with an enzyme-linked immunosorbent assay (ELISA) in a hospital setting using 225 patients, 6-24 months of age, who came with clinical symptoms suggestive of rotavirus diarrhoea. Of 225 specimens 120 (53%) were found positive with the ELISA compared with only 69 (31%) using the Rotascreen. Twelve specimens gave equivocal results with Rotascreen, 2 of which were positive and the remaining 10 were negative using the ELISA. Excluding the results of these 12 specimens by both the assays, the Rotascreen showed a specificity of 95% and sensitivity of 54%, in comparison with results in the ELISA. Rotascreen is a rapid and simple test with a satisfactory specificity but a low sensitivity.

Key words: Rotaviruses; Diarrhoea, Infantile, Diarrhoea, Acute; Enzyme-linked immunosorbent assay; Latex fixation tests; Diagnosis, Laboratory.

Introduction

Rotavirus has been recognised world-wide as the most important aetiological agent in infantile diarrhoea (1). It was first discovered by electron microscopy of a patient's stool. This is a laborious and expensive method for routine use. Various immunoassays (2) have been developed for this purpose and more recently latex agglutination slide tests have been developed and evaluated (3-7). Because it is difficult to identify rotavirus infection by clinical signs alone, there is a great demand for rapid and simple techniques to identify rotavirus in stool specimens for both clinical and epidemiological purposes. Such a test would also help to reduce the widespread misuse of antimicrobial drugs.

The aim of this study was to compare the sensitivity and specificity of a commercially available latex agglutination test, Rotascreen^R, with those of a commonly used enzyme-linked immunosorbent assay (ELISA) to detect rotavirus in stool specimens from children with acute diarrhoea.

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Materials and methods

This study was carried out at the Dhaka Treatment Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from September to December 1985. This time of the year is considered to be the peak season of rotavirus diarrhoea in Bangladesh (8). The study was approved by the Centre's Ethical Review Committee. Patients were selected from children with acute diarrhoea coming to the Treatment Centre. Children with clinically presumptive rotavirus diarrhoea were selected

according to the following criteria: age between 6 and 24 months, vomiting, and watery diarrhoea without blood of not more than seven days' duration. Patients whose stool samples were collected came mainly from the slum areas of Dhaka city. All of them were clinically examined by the same investigator (LG). A fresh stool sample was collected, part of which was stored frozen at −20°C and another part was used immediately in the latex slide agglutination test.

In this study we used the Rotascreen^R latex agglutination diagnostic kit (Mercia Diagnostics Ltd. Surrey, UK) which contains a test reagent (coated with antibodies against rotavirus), a negative control reagent, positive control and a buffer (formula not declared). The reagent batches used were given by manufacturer's code numbers: 014, 014A, 015, 016. The test was done according to the instructions given in the kit.

Briefly, a uniform suspension of a specimen of stool was prepared in buffer by shaking it in a tube. It was then allowed to stand for 5 min. After another shake, the specimen was centrifuged at approximately 2000 g for 10 min. From the supernatant 50 µl was dropped on two areas on the slide. The negative control and the test reagent were added. The test areas on the slide were stirred with a glass applicator and the slide was then gently rocked for 2 min before the test was read. All the tests in this study were done and read by the same investigator (LG). If there was any doubt about the result, the test was repeated at least once.

The frozen specimens were transported frozen to the Department of Virology, National Bacteriological Laboratory (NBL) in Stockholm for the ELISA. At the NBL, a stool specimen was prepared as a suspension in phosphate-buffered saline containing 0.05% Tween 20 and 0.5% Bovine Serum Albumin (PBS/T/B) at a concentration of 1:300. An ELISA as described by Svensson, *et al.* (9), was used to detect rotavirus group antigen as follows. Polystyrene microtitre plates (M 29 AR: Dynatech Ltd) were coated overnight at 4°C with guinea pig anti-rotavirus serum diluted 1:500 in 0.5 M sodium carbonate (pH 9.8) solution. After washing six times with PBS containing 0.05% Tween 20 (PBS/T) 100 µl of the stool suspension (see above) was added in duplicate wells and then incubated at 37°C for two hours. The plates were washed six times in PBS/T/B, followed by the addition of 100 µl rabbit anti-rotavirus diluted 1:1000 in PBS/T/B. (The rotavirus used for raising antibodies in the guinea pig and rabbit was taken from human source). After incubating for another two hours at 37°C the plates were washed six times before peroxidase-conjugated goat anti-rabbit IgG (Biorad, Western blotting grade) diluted 1:25,000 in PBS/T/B was added to each well. After a final incubation for 90 min. at 37°C the plates were washed and 100 mg/ml of 3,3 5 5-tetramethylbenzidine (TMB:Miles Lab, Naperville, USA) was added in 0.1 M sodium citrate-acetate buffer pH 6.0 with 1.3 mmol/l of fresh H_2O_2. The reaction was stopped with 2 M H_2SO_4. The absorbance was measured in a Titertek Multiscan spectrophotometer (Flow Ltd) set at a wavelength of 450 nM. The cut-off point was defined at reading level of 0.1. In addition, selected specimens were examined by electron microscopy.

Results

In this study a total of 225 patients were examined: their median age was 10.5 months and 151 (67%) were males. All patients suffered from dehydration of which 25% were of severe grade.

Rotavirus was detected by the ELISA in the stools of 120 patients (53%) and by the latex agglutination test in the stools of 69 (31%). There was no difference in the group of patients excreting rotavirus in comparison with the 105 patients not doing so, regarding age, sex, nutritional status, and degree of dehydration. Five infections, not detected by the ELISA, were diagnosed by the agglutination; these were confirmed by electron microscopy. The agglutination test also gave 12 equivocal results in which there was agglutination with both the test samples and the negative control; two of these were positive by the ELISA and checked by electron microscopy.

If the results of the 12 equivocal tests are excluded from the analysis, 118 infections were detected using the ELISA, among which 64 were found positive by the agglutination. Of the 95 samples which were shown by the ELISA not to contain rotavirus, 90 showed the same result by the agglutination test. Thus the

Rotascreen latex agglutination test had a specificity of 95% and a sensitivity of 54%.

Discussion

The Rotascreen test evaluated in this study is not the first latex agglutination test described. Other tests have been evaluated and compared with commonly used tests for rotavirus (3-7). The Rotascreen is a new test, thus the experience with its use is still limited. The results obtained in the study allow us to conclude that this latex agglutination test is not particularly sensitive when compared with an ELISA but is quite specific.

However, in spite of its limited sensitivity, the test may still be profitably used by a clinician in his office or hospital ward to detect more than 50% of the total rotavirus diarrhoea cases. The results also indicate that diarrhoea due to rotavirus is not reliably diagnosed by clinical features alone, as the virus was only detected in the faces of 56% of patients who had clinically suspected infections.

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