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Original Article

Utilization of a Latex Agglutination Test for the Rapid Detection of *Shigella* in Stool Specimens of Children With the Hemolytic Uremic Syndrome

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ABSTRACT

Background: The hemolytic uremic syndrome (HUS) is one of the important causes of acute renal failure in childhood. It is classified into diarrhea-associated and atypical cases. Verotoxin-associated HUS is the most common type of the disease. It is characterized by the sudden onset of hemolytic anemia with fragmented erythrocytes, thrombocytopenia, and acute renal failure after a prodromal illness of acute gastroenteritis usually with bloody diarrhea. In addition to specific strains of E. Coli, *Shigella dysentriae* type 1 can be an important cause of HUS. Cases with *Shigella dysentriae* have a higher mortality rate, and a greater incidence of complications and extra-renal manifestations compared with patients who have HUS related to E Coli. Therefore, it would be important to have a rapid way to detect *Shigella*-associated HUS, in order to anticipate its complications, and allow the administration of aggressive management to such patients.

Conventional stool cultures are labour-demanding and time-consuming, and therefore may not be the ideal method for the detection of certain stool pathogens in acute situations like the hemolytic uremic syndrome. A rapid, cost-effective and reliable method for the detection and identification of stool pathogens in stool specimens from these patients would be of great value.

Objectives: The aim of this work is to evaluate the utilization of a rapid latex agglutination test in comparison to conventional stool culture to determine the incidence of *Shigella dysentriae* among children with the HUS in Egyptian children, and to determine whether this test could be used as an alternative to conventional stool culture in this acute situation.

Methods: In this study, a latex agglutination test (Bactigen Salmonella/Shigella latex agglutination slide test; Wampole Laboratories, Cranbury, NJ) was evaluated as a test for the detection of Shigella antigens in Gram Negative Broth for the early and rapid detection of Shigella dysentriae infection in infants and children presenting with evidence of the hemolytic uremic syndrome.

Results: Utilization of conventional stool culture revealed that 3 of 17 cases of the HUS were positive for *Shigella dysentriae*, an incidence of 17 %. Utilization of the BSST latex agglutination test for Shigella antigen shows that 5 of 17 cases of the HUS were positive for Shigella dysentriae (29.5 %). Also, all 3 cases in which stool culture was positive for Shigella dysentriae were also positive by the BSST test, with no false negatives (sensitivity 100 %, positive predictive value 80%). However, of the 14 cases of HUS who were negative for Shigella by culture, two cases were positive for Shigella by the BSST latex agglutination test (Specificity 85 %, negative predictive value 100 %).

Conclusions: The BSST latex agglutination test is a useful method for the rapid detection of Shigella antigens in stool specimens from infants and children with the HUS. Due to its rapidity, it can provide a quick answer and allow the anticipation of the need for more aggressive management for cases of HUS associated with Shigella gastroenteritis. Its sensitivity appears to be excellent. While its specificity appears to be low, with a high incidence of false positive results. However, in such an acute situation, it is much more important not to miss a positive case than to over-diagnose a case with Shigellosis. Also, conventional stool culture can correctly identify the true pathogen in false positive cases.

INTRODUCTION

The hemolytic uremic syndrome is one of the important causes of acute renal failure in childhood. It is classified into diarrhea-associated and atypical cases[1]. Verotoxin-associated HUS is the most

common type of the disease. This exotoxin is thought to produce endothelial cell injury, initiating the process of microangiopathy, which results in hemolysis, platelet consumption and organ ischemia[2,3]. Clinically, HUS is characterized by the sudden onset of hemolytic anemia with fragmented erythrocytes, thrombocytopenia, and acute renal failure after a prodromal illness of acute gastroenteritis usually with bloody diarrhea. However, the diarrhea may not be bloody or may be absent in some cases. Most patients recover, and it is rare to see recurrences. Infections with *E. Coli* 0157 H7 are the most common causes of verotoxin-associated HUS. Other strains of *E. Coli* are less commonly found. In addition, infection with *Shigella dysentriae* type 1 can also be an important cause of HUS. Shiga-like toxins are related to Shiga toxin, the exotoxin produced by *Shigella dysentriae* type 1[4]. Cases with *Shigella dysentriae* have a higher mortality rate, a more severe leukemoid reaction, a disseminated coagulopathy, and an absence of mesangial lesions compared with patients who have HUS related to E. Coli[5]. It has been known that Shigatoxin produced by *Shigella dysentriae* causes neurological manifestations in association with Shigellosis. It has been suggested that the neurotoxin produced by Shigella causes neurological damage because of its effects on endothelial cells of the small vessels supplying the neurons[6]. The incidence of *Shigella dysentriae*-associated HUS has increased in certain locations[7]. HUS occurred within a week of the onset of diarrhea and had a mortality rate of 19%. Similarly high mortality rates are associated with *Shigella dysentriae*-associated HUS from India[8].

For these reasons, utilization of a rapid technique to detect Shigella-associated HUS would help us to anticipate its complications, and allow preparing for more aggressive management of such patients.

Conventional stool cultures are difficult and time-consuming, and therefore may not be the ideal method for the detection of certain stool pathogens in acute situations like the hemolytic uremic syndrome. A rapid, cost-effective and reliable method for the detection and identification of stool pathogens in stool specimens from these patients would be of great value. There has been much enthusiasm for the introduction of latex agglutination tests for the detection of Shigella antigens in selective enteric broths[9-11].

AIM OF THE WORK

The aim of this work is to evaluate the utilization of a rapid latex agglutination test in comparison to conventional stool culture to determine the incidence of *Shigella dysentriae* among children with the HUS in Egyptian children, and to determine whether this test could be used as an alternative to conventional stool culture in this acute situation.

SUBJECTS AND METHODS

In this study, a latex agglutination test (Bactigen Salmonella/Shigella latex agglutination slide test; Wampole Laboratories, Cranbury, NJ) was evaluated as a test for the detection of Shigella antigens in Gram Negative Broth for the early and rapid detection of Shigella dysentriae infection in infants and children presenting with

evidence of the hemolytic uremic syndrome. 17 children (6 males, 11 females) aged between 1-6 years were diagnosed as HUS clinically and by appropriate laboratory investigation from Cairo University Children's Hospital during the periods from March 1996-August 1997 and from August-October 2001. They were subjected to a full history and physical examination as well as appropriate laboratory investigations, including CBC with red cell morphology, platelet count, BUN, serum creatinine, stool analysis.

Stool specimens from these patients were tested utilizing 1- Conventional stool culture and 2- the Bactigen Salmonella/ Shigella latex agglutination test for the detection of Shigella antigens in Gram negative Broth inoculated with stool and rectal swab specimens. Stool specimens were inoculated on trypticase soy agar with 5% sheep blood, MacConkey (MAC) and Salmonella/Shigella (SS) agar. In addition, a CN broth was inoculated and incubated at 35 C for 4-5 hours prior to subculturing to MAC and SS. For the purpose of the study, the GN Broth was incubated further for an additional 12-24 hours. At the end of this additional incubation period, the BSST was performed according to the manufacturer's recommendations. Positive test results were considered with any 1+ or greater agglutination. When no agglutination occurred with any of the latex reagents, the specimen was considered negative for Shigella antigens. Non-specific reaction was recorded when 1+ or greater agglutination occurred with more than one reagent latex. Positive controls were also tested with patient specimens.

RESULTS

In this study, a latex agglutination test (Bactigen Salmonella/Shigella latex agglutination slide test; Wampole Laboratories, Cranbury, NJ) was evaluated as a test for the detection of Shigella antigens in Gram Negative Broth for the early and rapid detection of Shigella dysentriae infection in infants and children presenting with evidence of the hemolytic uremic syndrome. Stool specimens from these patients were tested utilizing 1- Conventional stool culture and 2- the Bactigen Salmonella/ Shigella latex agglutination test for the detection of Shigella antigens in Gram negative Broth inoculated with stool and rectal swab specimens.

As shown in Table 1, utilization of conventional stool culture revealed that 3 of 17 cases of the HUS were positive for *Shigella dysentriae*, an incidence of 17%. Utilization of the BSST latex agglutination test for Shigella antigen shows that 5 of 17 cases of the HUS were positive for *Shigella dysentriae* (29.5%). Also, as shown in table 1, all 3 cases in which stool culture was positive for *Shigella dysentriae* were also positive by the BSST test (sensitivity 100%, positive predictive value 80%). However, of the 14 cases of HUS who were negative for Shigella by culture, two cases were positive for Shigella by the BSST latex agglutination test (Specificity 85%, negative predictive value 100%).

All patients were subjected to a full history and physical examination as well as appropriate laboratory investigations, including CBC with red cell morphology, platelet count, BUN, serum creatinine, stool analysis. Table 2 summarizes the clinical

and laboratory characteristics at presentation of infants and children with HUS with and without Shigella gastroenteritis.

As shown, the age of infants with Shigella-associated HUS tended to be younger (range 1-2.5 years) than children with HUS not associated with Shigella (range 2-6 years). Also, of the 3 children with Shigella-associated HUS, one (33%) presented with an impaired level of consciousness, suggestive of CNS involvement with the microangiopathy. Only two of 14 (14%) children with HUS not associated with Shigella presented with mild drowsiness. Laboratory investigations are suggestive of a tendency towards lower platelet counts, as well as a higher prevalence of leukocytosis in children with Shigella-associated HUS (Table 2).

Table 1: Comparison of BSST and stool culture for the detection of *Shigella* in stool specimens from children with HUS

	Culture Results		
BSST results	**Positive for *Shigella***	**Negative for *Shigella***	**Total**
BSST positive	3	2	5
BSST negative	0	12	12
Total	3	14	17

Table 2: Clinical and laboratory characteristics of infants and children with HUS at presentation. Cases with and without Shigella gastroenteritis are compared

	Culture Results	
	Positive for *Shigella*	**Negative for *Shigella***
Clinical features		
Age	1 - 2.5 yrs	2 - 6 yrs
Sex	1 male , 2 females	5 males, 9 females
Bloody stools	3/3	10/14
Fever	2/3	8/14
Oliguria	3/3	14/14
Pallor	3/3	13/14
Drowsiness	1/3	2/14
Seizures	0/3	0/14
Lab features (range)		
Hemoglobin	4 - 9 gm%	3 - 9 gm%
Platelets	10 - 60,000	50 - 120,000
Leukocytosis	3/3	5/14
BUN	60 - 120 mg/dl	54 - 115 mg/dl
Creatinine	0.9 - 1.1 mg/dl	0.7 - 2.1 mg/dl

DISCUSSION

The hemolytic uremic syndrome is one of the important causes of acute renal failure in childhood. It is classified into diarrhea-associated and atypical cases[1]. Verotoxin-associated HUS is the most common type of the disease. It is characterized by the sudden onset of hemolytic anemia with fragmented erythrocytes, thrombocytopenia, and acute renal failure after a prodromal illness of acute gastroenteritis usually with bloody diarrhea.

In addition to specific strains of E. Coli, *Shigella dysentriae* type 1 can be an important cause of HUS. Cases with *Shigella dysentriae* have a higher mortality rate, a more severe leukemoid reaction, a disseminated coagulopathy, and an absence of mesangial lesions compared with patients who have HUS related to E. Coli[5]. Shigella infections may also have a higher incidence of associated extra-renal, eg neurological manifestations[6]. For these reasons, utilization of a rapid technique to detect Shigella-associated HUS would help us to expect its complications, in order to be prepared for more aggressive management of such patients.

Conventional stool cultures are time-consuming, and therefore may not be the best method for the detection of certain stool pathogens in acute situations like the hemolytic uremic syndrome. A rapid and reliable method for the detection and identification of pathogens in stool specimens from these patients would be very useful. Previous reports describe the introduction of latex agglutination tests for the detection of Shigella antigens in selective enteric broths[9].

In this study, a latex agglutination test (Bactigen Salmonella/Shigella latex agglutination slide test; Wampole Laboratories, Cranbury, NJ) was evaluated as a test for the detection of Shigella antigens in Gram Negative Broth for the early and rapid detection of Shigella dysentriae infection in a group of Egyptian infants and children presenting with evidence of the hemolytic uremic syndrome.

Utilization of conventional stool culture revealed that 3 of 17 cases of the HUS were positive for *Shigella dysentriae* , an incidence of 17%. Utilization of the BSST latex agglutination test for Shigella antigen shows that 5 of 17 cases of the HUS were positive *for Shigella dysentriae* (29.5%). Also, as shown in table 1, all 3 cases in which stool culture was positive for Shigella dysentriae were also positive by the BSST test and therefore no false negatives were encountered (sensitivity 100%; positive predictive value 80%). However, of the 14 cases of HUS who were negative for *Shigella* by culture, two cases were positive for Shigella by the BSST latex agglutination test (Specificity 85%; negative predictive value 100%).

In conclusion, the BSST latex agglutination test is a useful method for the rapid detection of Shigella antigens in stool specimens from infants and children with the HUS. Due to its rapidity, it can provide a quick answer and allow the anticipation of the need for more aggressive management for cases of HUS associated with Shigella gastroenteritis. Its sensitivity appears is excellent, but its specificity appears to be lower, with a high incidence of false positive results. However, in such an acute

situation, it is much more important not to miss a positive case than to over-diagnose a case with Shigellosis. Also, conventional stool culture will correctly identify the true pathogen in false positive cases. Further studies will be required to assess the usefulness of this test and other rapid tests under these circumstances.

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