Serum antibodies to Verotoxin-producing *Escherichia coli* (VTEC) strains in patients with haemolytic uraemic syndrome

K. KISHORE, A. RATTAN†, A. BAGGA*, R. N. SRIVASTAVA*, N. MANJU NATH and SHRINIWAS

Departments of Microbiology and *Paediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India

Summary. Serological evidence of infection with verotoxin-producing *Escherichia coli* (VTEC) was sought in 28 patients suffering from haemolytic uraemic syndrome (HUS) and 25 age- and sex-matched controls. ELISA was used to detect anti-lipopolysaccharide (LPS) antibodies to *E. coli* strains O157, O111, O26 and NCTC 10418, a non-VTEC strain, and *Shigella dysenteriae* O1. Sera from 19 of the HUS patients but from none of the 25 controls had significant antibody levels to the verotoxin-producing bacteria. Sera from 13 patients reacted with only one LPS of the four verotoxin-producing bacteria; sera from six reacted with more than one LPS antigen but not with LPS of *E. coli* NCTC 10418. Paired sera taken 2–3 weeks apart were obtained from 20 HUS patients; 14 of these had high levels of antibody in the acute phase sample. Analysis of antibody levels in the convalescent sera showed that one patient had an increase, one was unchanged and 12 patients had a decrease in antibody to the verotoxin-producing bacteria.

Introduction

Haemolytic uraemic syndrome (HUS) is the simultaneous appearance of acute renal failure, microangiopathic haemolytic anaemia and thrombocytopenia, arising predominantly in infants and young children following a prodrome of diarrhoea.¹ In India, HUS is the commonest cause of acute renal failure in children.²

Since 1983, when Karmali *et al.*³ first indicated the association of verotoxin-producing *Escherichia coli* (VTEC) with HUS, research has concentrated principally on the isolation of VTEC from patients suffering from HUS. In a prospective study, Karmali *et al.*⁴ reported infection with VTEC in 74% of cases of HUS. Similar findings were reported from other parts of the world.^{5–7}

In India, HUS has been reported from Delhi,^{8,9} Srinagar,¹⁰ Bombay,¹¹ Calcutta,^{12,13} Nagpur,¹⁴ Madras¹⁵ and Vellore.¹⁶ However, our attempts to isolate VTEC from the stools of HUS patients were unsuccessful.¹⁷ Serological tests for antibodies to lipopolysaccharide (LPS) of *E. coli* O157 as an adjunct to culture have been reported to be valuable.^{18–20} Therefore, we examined sera from HUS patients for the presence of antibodies to LPS derived from *E. coli* strains of serotypes *E. coli* O157, O111 and O26—which have been reported to be high level producers of

verotoxin—as well as *Shigella dysenteriae* O1 and *E. coli* NCTC 10418, a non-VTEC strain.

Materials and methods

Patients and serum samples

A patient with HUS was defined as a previously healthy child who had developed acute renal failure, thrombocytopenia, and haemolytic anaemia following a diarrhoeal or dysenteric illness;²¹ 28 HUS patients were studied. Paired serum samples taken 2–3 weeks apart were obtained from 20 patients. Single samples were obtained from the remaining eight patients and from 25 healthy controls matched for age and sex. The mean interval between onset of diarrhoea and collection of serum was 17 days (range 8–32 days).

LPS antigens

LPS antigens were prepared from *E. coli* strains of serotypes O157 (from Dr L. W. Riley, WHO Consultant, CDC, Atlanta, USA), O111 and O26 (from the Central Research Institute, Kasauli), *S. dysenteriae* and *E. coli* NCTC 10418 (from our stock cultures) by the method of Westphal and Jann.²²

ELISA for LPS antibodies

ELISA was performed by coating and incubating polystyrene plates overnight at 4°C with the optimum

Table. ELISA for LPS antibodies in sera of HUS patients

Patient no.	OD ₄₉₂ with LOPS of <i>E. coli</i> serotype									
	O157		O111		O26		<i>S. dysenteriae</i> O1		<i>E. coli</i> NCTC 10418	
	Acute	Conv.	Acute	Conv.	Acute	Conv.	Acute	Conv.	Acute	Conv.
1	1.2	0.8	1.3	0.8	1.5	0.7	1.2	0.7	0.8	0.7
2	1.3	0.8	2.3	0.8	1.5	0.8	2.4	0.7	0.6	0.8
3*	2.3*	1.0	2.6*	1.9	2.6*	1.0	2.6*	2.3*	0.9	0.7
4	0.5	0.6	0.8	0.6	0.5	0.8	0.5	0.8	0.7	0.7
5*	2.3*	0.8	0.3	0.7	0.7	0.8	0.5	0.8	0.8	0.6
6*	0.9	1.1	0.5	2.1	1.8	2.1*	0.7	2.1	0.6	0.8
7*	0.3	...	2.5*	...	2.5*	...	2.6*	...	0.8	...
8*	2.6*	1.2	1.1	1.1	2.1*	1.2	1.3	1.2	0.9	0.9
9*	2.0	1.0	2.5*	0.9	2.7*	1.1	2.4	1.0	0.7	0.6
10*	2.6*	...	0.6	...	1.4	...	0.7	...	0.5	...
11*	1.1	...	1.4	...	1.9*	...	1.9	...	0.8	...
12	1.7	...	1.0	...	1.4	...	1.3	...	0.9	...
13*	1.8	...	1.5	...	2.6*	...	2.3	...	1.1	...
14*	1.7	1.1	1.6	2.1	2.1*	2.0*	2.7*	2.7*	0.9	0.9
15	0.7	1.0	0.4	1.5	1.4	1.0	0.7	1.3	0.5	0.3
16	1.2	0.9	1.6	0.9	0.4	1.0	1.8	1.2	0.6	0.8
17*	1.4	1.2	0.1	1.1	2.5*	1.2	0.8	1.4	0.5	0.3
18*	1.3	0.8	0.9	0.7	2.2*	0.8	1.4	0.8	0.4	0.7
19*	0.8	0.7	0.4	0.8	2.2*	0.8	2.3	0.8	0.7	0.6
20	0.9	...	1.2	...	1.2	...	1.5	...	0.9	...
21*	1.9	0.8	1.6	0.8	1.4	0.7	2.6*	0.7	1.2	0.8
22	0.2	0.1	0.2	0.1	0.3	0.6	0.4	0.8	0.3	0.3
23*	0.7	0.9	0.9	1.0	2.3*	1.0	2.0	1.0	0.5	0.5
24*	1.9	0.9	2.3	0.9	0.1	1.1	2.6*	1.1	0.8	0.9
25*	1.4	0.8	0.9	0.7	0.6*	0.8	2.6*	0.7	1.0	1.1
26	1.1	...	1.8	...	1.2	...	1.8	...	1.2	...
27*	1.8	1.7	1.9	0.4	2.7*	0.6	2.1	0.6	0.9	0.6
28*	1.9	...	0.9	...	2.2*	...	2.1	...	0.7	...

..., No specimen.

*Patients and sera with significant antibody levels to the verotoxin-producing bacteria.

dilution of each LPS in carbonate-bicarbonate buffer pH 9.6. After washing the plates with phosphate-buffered saline (PBS), pH 7.2, the uncoated sites were blocked by incubation with bovine serum albumin 1 % in PBS for 30 min at 37°C. After washing the plates in PBS and blotting dry, serum samples diluted 100-fold in PBS were added in duplicate and incubated at 37°C for 30 min. After further washing and drying, anti-human γ globulin conjugated with horseradish peroxidase (Dakopatts, Denmark; diluted 1 in 5000) was added and incubated at 37°C for 30 min. After washing, *o*-phenylene diamine (Sigma) and H₂O₂ were added to each well. Colour development was stopped after 20 min and the absorbance at 492 nm (OD₄₉₂) was measured in a microelisa reader (Titertek).

Results

The ELISA results for sera from HUS patients are shown in the table. The K value, i.e., the mean + 2 SD of the OD₄₉₂ ELISA readings of the control sera for each organism, was calculated. The K values of control sera to *E. coli* strains of serotypes O157, O111, O26 and *S. dysenteriae* O1 were 1.12, 1.43, 0.84 and 1.48 respectively. Antibodies to verotoxin-producing bacteria were said to be present in significant amounts in HUS patients if the OD₄₉₂ ELISA reading for an

organism was > 1.0 after subtraction of the K value. The HUS patients with significant levels of antibody to the verotoxin-producing bacteria are shown (*) in the table.

Of the 28 HUS patients, 19 had significant antibody levels to one or more of the verotoxin-producing bacteria. The ELISA reading against the non-verotoxin-producing *E. coli* strain NCTC 10418 of the sera of HUS patients was not significantly different from that of the controls. Sera from 13 patients reacted with only one LPS antigen and sera from six reacted with more than one antigen. Serum from one patient reacted with LPS from all four verotoxin-producing strains but not with *E. coli* NCTC 10418, another reacted with three LPS antigens, and sera from four patients reacted with two LPS antigens of verotoxin-producing bacteria.

Paired sera were obtained from 20 HUS patients; 14 of these had high levels of antibodies to LPS in the acute phase sample. One patient showed an increase in antibody, another no change and in 12 patients there was a significant decrease in antibody in the convalescent phase serum samples.

Discussion

The detection of VTEC or free faecal VT, or both, has proved essential in the diagnosis and epidemiology

of HUS. Unfortunately, the bacteria that cause the disease and the toxins they produce are detectable in faecal specimens for only a limited time following the onset of disease.^{23,24} Tarr *et al.*²⁵ studied HUS cases prospectively and reported that VTEC could be isolated in every case if the samples were collected and examined within the first 2 days of diarrhoea, but the isolation rate fell to 33.3% if the samples were collected and examined after the first week of the onset of diarrhoea. Others have made similar observations.²⁶ None of our cases had a history of less than 1 week of onset of diarrhoea.

Because *E. coli* serotypes O157, O111, and O26 appeared to be the major VTEC strains, and since *S. dysenteriae* O1 had been associated with HUS cases from Vellore and West Bengal, we sought to detect antibodies to them. The inclusion of the non-VTEC strain NCTC 10418 permitted the detection of non-specific or cross-reaction antibodies. High OD values were given by sera to one or more LPS antigens from the four verotoxin-producing bacteria were found in sera from 19 (67.8%) of 28 HUS patients. Whereas sera from 13 patients reacted with only one antigen, six reacted with more than one LPS. However, all these sera had only low levels of anti-LPS antibodies against *E. coli* NCTC 10418. Chart *et al.*^{18,19} reported that while some HUS serum samples from patients with *E. coli* O157 cross-reacted with outer-membrane proteins from other serotypes of *E. coli*, there was no cross-reaction with the LPS antigens obtained from serotypes O111, O119, O127, O142 and O26. HUS patients' sera in their study group did not react with the H7 flagellar antigen. Cross-reaction between the O antigen of *E. coli* O157 and *Brucella abortus* and *Yersinia enterocolitica* has also been reported.²⁷ Toth *et al.*²⁸ investigated the presence of cross-reacting plasmid-coded antigens in *E. coli* O157:H7 and other *E. coli* strains. They reported that plasmid-containing strains of *E. coli* O157:H7 secreted two proteins, of

82- and 92-kDa, which were absent in plasmid-cured strains. When rabbit antisera were raised and made monospecific against these proteins, all 15 wild-type *E. coli* O157:H7 and 10 VTEC-strains of other serotypes reacted positively in a direct ELISA, whereas 13 of 14 enterotoxin-producing *E. coli*, all of 24 enteroinvasive *E. coli* strains and all 16 *E. coli* strains isolated from healthy persons did not.

The fact that sera from HUS patients showed high OD values against LPS from VTEC strains but low OD values against LPS of *E. coli* NCTC 10418 suggests that patients react to an epitope common to VTEC but not present in non-VTEC strains. Sera from six patients reacted with *S. dysenteriae* O1 LPS. It was observed that the mean OD to *S. dysenteriae* O1 was quite high in control sera, attesting to the endemicity of *S. dysenteriae* O1 in the region. Cross-reaction with VTEC LPS was observed in four HUS patients.

Analysis of antibody levels in paired sera revealed a rise in one case, no change in another, but a fall in 12 patients. This would attest to the fact that the patients usually came in the later stages of the disease to our hospital which is a tertiary care centre. This may be one of the main reasons for our failure to isolate VTEC from the stool samples submitted from these patients, as well as our inability to demonstrate the presence of free faecal verotoxin in both stool filtrates and polymyxin B extracted colony sweeps from primary culture plates.¹⁷

On the basis of serological evidence, we conclude that infection with *E. coli* O157, O111, and O26 strains occurs in HUS cases in India. *E. coli* O26 appears to be the commonest of the three serotypes.

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