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Vi serology in screening of typhoid carriers: improved specificity by detection of Vi antibodies by counterimmunoelectrophoresis

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SUMMARY

A purified soluble Vi antigen was used in counterimmunoelectrophoresis (CIE), passive haemagglutination (HA) and solid phase radio-immunoassay (SPRIA) for detection of serum Vi antibody. Serum Vi antibody was detected in 13 of 14 chronic typhoid carriers by both CIE and HA. SPRIA studies showed that Vi antibodies in sera of these carriers were mainly of the IgG class. Successful treatment with amoxycillin in one carrier, who initially showed a positive Vi test, resulted in a reversion in 6 months to seronegativity as measured by Vi CIE. However, Vi antibody was also detected in 10 sera (3%) from 329 control subjects by HA but not by CIE. When the CIE assay was applied to 1030 serum specimens obtained from hospitalized patients, it was able to detect one typhoid carrier, but one (0·1%) non-carrier also gave a positive Vi CIE test. It is thus concluded that Vi CIE is more specific and no less sensitive than Vi HA for the detection of the typhoid carrier state. The finding by SPRIA that the anti-Vi antibodies present in typhoid carriers' sera were mainly of the IgG class further justified the use of CIE as a precipitation test for their detection.

INTRODUCTION

It has been generally recognized that most chronic typhoid carriers have serum Vi antibodies. Therefore, for years, Vi bacterial agglutination or Vi haemagglutination tests have been used for screening for the typhoid carrier state. However, the main problem with the Vi agglutination test was insufficient specificity (Public Health Laboratory Working Party, 1961; Bokkenheuser, Smith & Richardson, 1964; Cooper & Singh, 1965). Recently, Nolan *et al.* (1980) reported that, using a passive haemagglutination assay with a purified Vi antigen, they were able to detect serum Vi antibody in 22 (71%) of 31 typhoid carriers studied but in none of 22 control subjects. The number of subjects in their control group was however too small to evaluate the specificity of their Vi haemagglutination assay.

In an attempt to improve the specificity of these Vi agglutination tests, we have tried a modified Vi HA test by agglutination against mercaptoethanol-treated sera and a Vi fluorescent antibody test (Chau & Chan, 1976). The false positive rate with the former test was reduced to 3% and with the latter to 1·5% as compared

with an 8% false positive rate with a conventional Vi haemagglutination test. These tests were however too tedious to be of practical value for large scale screening for typhoid carriers. Later, during our study of the protein antigens of *Salmonella typhi* extracted by Barber's method (Barber & Eylan, 1976), we noticed that sera from six typhoid carriers all reacted with the Barber's extract to give precipitation lines on counterimmunoelectrophoresis (CIE) or to give a distinct anodally migrating precipitation line on immunoelectrophoresis (Chau, Tsang & Ng, 1980). This antigenic component was later found to be immunologically identical with the Vi antigen, i.e. either it was a Vi antigen by itself or a Vi–protein complex. This prompted us to evaluate CIE as a new assay for serum Vi antibody in the detection of the typhoid carrier state. The results are reported here.

MATERIALS AND METHODS

Typhoid carriers and control subjects

Fourteen faecal typhoid carriers, eight females and six males aged 40–72, were included in this study. All the carriers were asymptomatic and with no history of previous typhoid fever except four with gallstones. The duration of the known carrier state was difficult to ascertain but the carrier state was re-confirmed before this study by repeated positive cultures and in eight carriers by positive bile cultures as well.

Serum specimens were collected from these carriers. Three carriers completed a course of antibiotic therapy with amoxycillin 1 g four times a day for 1 month. Serum specimens were obtained and stool cultures were done 2 weeks after termination of antibiotic therapy. In one resolved carrier, serum and stool specimens were further collected at 1, 3 and 6 month intervals.

Control serum specimens were obtained from 329 hospitalized patients over the age of 40. No data about the history of previous typhoid fever or typhoid vaccination were available from these control subjects as the sera were sent to the laboratory mainly for serological tests for syphilis or for other investigations. For surveillance of typhoid carriers on a larger scale using the CIE test, 1030 serum specimens obtained from adult patients sent to the clinical chemistry laboratory for biochemical investigations were examined. Three successive stool and urine cultures were done on patients who had positive Vi antibody tests, i.e. a Vi HA titre greater than 40 or a positive Vi CIE test.

Methods for bacteriological investigation

Faecal specimens or centrifuged urine sediment were inoculated onto SS and MacConkey agars and into strontium selenite broth (Chau & Forrest, 1972), from which subculture on to SS agar was done after overnight incubation. Bile specimens were inoculated onto SS, MacConkey and blood agar plates directly. The identity of *S. typhi* was confirmed by standard biochemical and serological tests.

Serological tests for Vi antibodies

Sera were separated by centrifugation, portioned, and stored at −20 °C until assayed. Vi positive and negative control sera were always included in all the Vi antibody tests.

The purified Vi antigen was prepared by the ethanol–cetavlon mild precipitation method (Wong & Feeley, 1972) from a strain of *S. typhi*, which was freshly isolated from the blood culture of a typhoid patient and had well-developed Vi antigen. The same purified Vi antigen was used for the sensitization of human O erythrocytes in Vi HA, for coating of the wells of microtitre plates in SPRIA and for Vi CIE test.

Vi bacterial agglutination and Vi HA were carried out according to procedures described previously (Chau & Chan, 1976). Human group O erythrocytes were used in the haemagglutination assay. Vi HA titres of 40 or greater and Vi bacterial agglutination titres of 10 or greater were considered to be positive.

CIE was done according to the method used previously (Tsang & Chau, 1981) except that Vi antigen was used at a concentration of 10 μg/ml as the antigen and electrophoresis was carried out for 2 h at 3 mA per slide. Results were not read immediately after electrophoresis but after leaving the slides at 4 °C overnight and washing in 0·85% saline for 2 h.

SPRIA was done according to the procedure described elsewhere (Tsang *et al.* 1981) and briefly as follows: (i) purified Vi antigen at 100 μg/ml was coated in 50 μl vol. to wells in a U-type microtitre tray (Cooke Engineering Co., Alexandria, Va., U.S.A.); (ii) after reacting with sera from typhoid carriers or normal controls, 40 ng ^{125}I-labelled purified anti-human IgA (anti-alpha), -IgG (anti-gamma) and -IgM (anti-mu) antibodies were added in 50 μl vol. to each appropriate well; (iii) RIA antibody titre was arbitrarily chosen as the dilution of serum at which 2 ng of radio-labelled anti-human antibodies were bound.

RESULTS

If a Vi HA titre of 40 was taken as positive, then 13 (93%) of the 14 typhoid carriers had Vi antibodies in their sera, the same results were obtained when Vi antibodies were assayed by CIE. SPRIA studies revealed that the 13 positive sera all contained high Vi antibody titres in the IgG class (Table 1). By bacterial Vi agglutination, however, three of these 13 carriers showed serum Vi antibody titres less than 5, i.e. below the diagnostic level if a titre of 10 was taken as positive. Typhoid H and O agglutinins (titre equal to or greater than 80) were present respectively in the sera of nine and five typhoid carriers.

Three typhoid carriers completed a course of antimicrobial therapy with amoxycillin 1 g four times a day for 1 month. One female carrier was cured as confirmed by repeated successive negative stool cultures performed after termination of antimicrobial therapy at intervals of 2 weeks, and 1, 3 and 6 months. Vi antibody in her sera was no longer detectable by CIE 6 months after termination of therapy.

Table 1. *Detection of Vi antibodies by radioimmunoassay (RIA), haemagglutination (HA), bacterial agglutination (BA) and counterimmunoelectrophoresis (CIE) in sera of typhoid carriers*

	Serum Vi antibodies					
	RIA titre of antibodies of the					
Typhoid carrier	IgG-class*	IgA-class	IgM-class	HA titre	BA titre	CIE
1	4299	89	66	2560	80	+
2	1374	41	< 10	320	< 5	+
3	11885	32	< 10	2560	160	+
4	1349	20	< 10	1280	40	+
5	5843	200	68	640	20	+
6	7827	34	< 10	640	< 5	+
7	10560	40	< 10	1280	160	+
8	1720	56	< 10	640	20	+
9	282	93	< 10	20	10	−
10	1586	40	< 10	80	< 5	+
11	2780	27	< 10	320	20	+
12	6174	106	36	640	80	+
13	3584	38	< 10	640	40	+
14	1280	136	52	160	40	+

* The significant titre for Vi antibodies of the IgG class as measured by RIA was defined as equal to or greater than 1000.

Table 2. *Detection of serum Vi antibodies by HA and CIE in typhoid carriers and non-typhoid control subjects*

		Number of patients				
		with Vi HA titre of				with positive
Category	No.	< 40	40–80	160–320	640–2560	Vi CIE
Typhoid carriers	14	1	1	3	9	13
Control subjects	329	319	8	1	1	0

In the control group, 10 (3%) of the 329 patients gave serum Vi HA antibody titres equal to or greater than 40, but were all negative by CIE (Table 2). Repeated stool and urine cultures from these ten patients, and bile cultures in two patients, were negative for *S. typhi*. SPRIA done on these Vi antibody positive sera (as shown by HA) indicated that the Vi antibodies present were mainly of lower titres as compared to those in sera of typhoid carriers, and they were mainly of the IgM class (Table 3).

By bacterial Vi agglutination, 69 (21%) of the 329 control sera showed Vi antibody titres equal to or greater than 10. Since the sensitivity and specificity of this test in the detection of typhoid carriers were far from satisfactory, its use was abandoned in further studies.

Table 3. *Detection of Vi antibodies by radioimmunoassay (RIA), haemagglutination (HA) and counterimmunoelectrophoresis (CIE) in sera of non-carriers*

	Serum Vi antibodies				
		RIA titre of antibodies of the			
Serum from non-carriers	HA titre	IgG-class	IgA-class	IgM-class	CIE
1	160	514	17	416	—
2	40	87	10	134	—
3	80	27	< 10	346	—
4	80	68	126	300	—
5	320	607	61	575	—
6	80	460	< 10	297	—
7	40	19	< 10	147	—
8	40	271	86	178	—
9	80	29	43	251	—
10	160	826	29	68	—

Based on the above encouraging results, an attempt was made to screen for typhoid carriers by CIE. A total of 1030 patients' serum specimens collected from the clinical chemistry laboratory were examined by CIE for the presence of Vi antibodies. Serum specimens from two patients, one with biliary tract infection and the other with gallstones, were positive for Vi antibody. Stool and bile cultures revealed that the patient with gallstones was a carrier of *S. typhi* while the other was not. Thus, in this series of study, the false positive rate for the detection of typhoid carriers by CIE was only 0·1 %.

As serum in which the presence of Vi antibody was demonstrated by CIE was obtained from a non-carrier with biliary tract infection, we were interested to see whether other patients with biliary tract infection who were not typhoid carriers were also likely to have serum Vi antibodies. We therefore examined sera obtained from 20 patients over the age of 40 with biliary tract infection or biliary carcinoma. Four of these patients were found to have Vi HA titres $\geqslant 40$, and two of these four had Vi antibodies in their sera detectable by CIE. Stool, urine and bile cultures performed for all these 20 patients were negative for *S. typhi*. However, a strain of *Escherichia coli* which possessed Vi antigen as indicated by agglutination against a diagnostic anti-Vi antiserum (Wellcome Research Lab., Beckenham, England) was found in the bile culture from one of the two patients with positive Vi CIE test. It appeared that patients with biliary tract infection did give a higher proportion (2 in 20) of false positives than the general population if Vi CIE was used as a screen for typhoid carriers.

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DISCUSSION

For any given serological test, standardizaton of the procedure to obtain reproducible results is important, but in the case of bacterial Vi agglutination, it is difficult because Vi antigen is easily released from the bacterial cell surface. This is not unexpected since the extraction of Vi antigen from bacterial cells is normally done by initially shaking whole bacterial cells in 0·15 M NaCl solution (Landy & Lamb, 1953; Baker *et al.* 1959; Wong & Feeley, 1972). We have demonstrated the presence of such released soluble Vi antigen from the supernatant of different batches of Vi bacterial cell suspensions using a precipitation reaction against a commercial anti-Vi antiserum. Thus, batch-to-batch variation of bacterial Vi antigen suspensions is almost inevitable and standardization of the antigen preparation is rather difficult. The antigen used for Vi haemagglutination comprises erythrocytes sensitized with purified Vi antigen. This greatly improves the specificity of the test and eases the problem of standardization. However, there is still a significant percentage of false-positive reactions. A possible explanation is that as an agglutination test HA favours greatly the detection of antibodies of the IgM class. Vi antibodies of the IgM class were deficient in typhoid carriers' sera while present in sera of some of the non-carriers, e.g. 3% as indicated in this series of study (Table 2). CIE as a precipitation test, on the other hand, favours the detection of antibodies of the IgG class, which were the predominant Vi antibodies present in typhoid carriers' sera. Although it has been suggested that Vi and O antibodies in typhoid carriers' sera were mainly of the IgA class (Karolček, Draskovicva and Ciznar, 1975) or of both the IgA and IgG classes (Chernokhvostova *et al.* 1969), our results indicated that these antibodies were mainly of the IgG class (Table 1).

Vi CIE test could be of practical value not only in large scale screening but also in the routine surveillance of typhoid carriers, e.g. among family members of a typhoid patient. This is because the Vi antigen solution is stable and the procedure can be easily standardized. In our laboratory, a solution of purified Vi antigen stored for 1 year at 4 °C remained stable in regard to its antigenic potency. More importantly, the Vi CIE test is no less sensitive and much more specific than the Vi HA test. The Vi CIE test might also have a role in the evaluation of antimicrobial therapy for typhoid carriers, although in our hands only one out of three carriers was successfully cured by antimicrobial chemotherapy.

Judged from the results of measuring the IgG-class Vi antibodies in typhoid carriers' sera by RIA, it is reasonable to suggest that enzyme-linked immunosorbent assay (ELISA), which unlike RIA uses no radioactive material, should be better than Vi CIE for screening for the typhoid carrier state because ELISA is able to measure quantitatively the levels of immunoglobulin class-specific Vi antibodies: the presence of the IgG class serum Vi antibodies at high levels indicates the typhoid carrier state. However, ELISA requires sophisticated instruments and expensive reagents while CIE does not. Thus the choice between these two tests will depend largely on feasibility.

It is interesting to find that a higher proportion of non-carriers with biliary tract

infections had Vi antibodies in their sera. The presence of serum Vi antibodies in these patients might be due to infection in the biliary tract by enteric bacteria, e.g. *E. coli*, possessing Vi antigen. Bile might have a role in the release of Vi antigen from the bacteria, or in the presentation of Vi antigen to the host, to elicit a humoral immune response against this antigen.

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