DIAGNOSTIC MICROBIOLOGY

Rapid diagnosis of typhoid fever by detection of Barber protein and Vi antigen of *Salmonella* serotype Typhi

MANISHA PANDYA, PARUKUTTY PILLAI and MANORAMA DEB

Department of Microbiology, Lady Hardinge Medical College, New Delhi 110001, India

Summary. Co-agglutination (coagg) and latex agglutination (LA) tests were used for the detection of *Salmonella* serotype Typhi Vi and Barber protein (BP) antigens in sera from five groups of individuals (A–E). Group A consisted of 30 blood culture-positive cases of typhoid fever and group B consisted of 30 suspected cases of typhoid fever who had sterile blood cultures but positive Widal tests. Thirty cases of pyrexia of unknown origin (PUO) were placed in group C, while group D consisted of 15 cases of septicaemia caused by organisms other than *Salmonella* serotype Typhi. Group E comprised 50 normal healthy individuals with no history of typhoid fever or TAB vaccination in the previous 5 years. The Vi-LA test performed best with 96.7% of group A sera and 90% of group B sera giving positive results. No false positive results and only 2.58% false negative results were obtained with this test. Considering patients with positive blood culture results or positive Widal tests as true positives, the sensitivities of the Vi-LA, BP-LA, Vi-coagg and BP-coagg tests were 93.3, 91.7, 83.3 and 86.7%, respectively. The specificities of these tests were 100, 98.5, 98.5 and 98.5%, respectively. It is suggested that the Vi-LA test can be used for the rapid and early diagnosis of typhoid fever.

Introduction

The diagnosis of typhoid fever in endemic areas mainly depends on the isolation of *Salmonella* serotype Typhi from blood culture. This has certain disadvantages. Many cultures are falsely negative due to prior antibiotic therapy and results take at least 48–72 h.¹ The currently available serological method for diagnosis of typhoid fever, the Widal test, requires both acute and convalescent sera and the results are often found to be unreliable in endemic areas.^{2,3} Therefore, for the purposes of rapid diagnosis it is preferable to detect Typhi antigen in the body fluids of patients with typhoid fever.^{4–8} The present study was undertaken to evaluate a co-agglutination (coagg) test and a latex agglutination (LA) test for the detection of soluble Typhi Barber protein antigen (BP)⁹ and Vi antigen in patients' sera.

Materials and methods

Patients and controls

The typhoid fever group consisted of 60 patients with clinical and laboratory evidence of typhoid fever.

The clinical diagnosis was based on the presence of unremitting fever for > 5 days and the presence of one or more of the following symptoms: systemic toxicity; fatigue; abdominal symptoms; transient rash; anorexia; and a palpable spleen. Routine blood cultures and a Widal test with paired sera with a 1-week interval between collections were done in all 60 patients. A four-fold rise in titre of antibody to Typhi "O" and "H" antigens was considered as a positive Widal test result. Thirty blood culture-confirmed typhoid cases were assigned to group A and thirty blood culture-negative but Widal test-positive cases to group B. Group C consisted of 30 cases of pyrexia of unknown origin (PUO) which were bacteriologically sterile on blood culture and showed negative Widal tests with paired sera. The febrile control group (group D) comprised 15 bacteriologically confirmed cases of other non-typhoidal infectious diseases which also showed negative Widal tests. All these 15 cases were septicaemic and blood cultures yielded *Escherichia coli*, *Klebsiella* spp. (five each), *Salmonella* Paratyphi A (three) and *Salmonella* Typhimurium (two). Group E consisted of 50 normal healthy individuals with no past history of typhoid fever or TAB vaccination in the previous 5 years. Blood (5 ml) was collected from all subjects and the serum was separated and stored at –20°C until tested. All agglutination tests were

performed on sera collected within the first week after onset of fever.

Protein antigens

Typhi BP was prepared from *Salmonella* serotype Typhi strain 0901 (National Phage Typing Centre, Lady Hardinge Medical College, New Delhi, India) by the method described by Barber *et al.*⁹ Briefly, harvested, washed and acetone-dried cells were extracted with veronal buffer (pH 8.4) and BP was purified by precipitation with trichloroacetic acid 10%. The precipitate was washed, dissolved, dialysed against phosphate-buffered saline (PBS), pH 7.2, lyophilised and stored at 4°C until used. Protein antigens were also prepared from clinical isolates of *Salmonella* Paratyphi A, *Salmonella* Typhimurium, *E. coli* and *Klebsiella pneumoniae* by the same procedure. The protein content of these antigen preparations was determined by the method of Hartree.¹⁰

Preparation of rabbit antisera to BP

Antiserum to BP was raised in rabbits and subsequently purified.¹¹ Adult albino rabbits (c. 2 kg in weight) were immunised in the two front foot pads and at four other subcutaneous sites with 1 mg of BP. One month later trial bleeding was done and antibody titres were measured by double immunodiffusion against Typhi BP. The animals with high antibody titres (≥ 2000) were bled and antisera were pooled. An equal volume of saturated ammonium sulphate (4°C) was added to pooled rabbit antisera to precipitate the globulin fraction and anti-BP immunoglobulin (IgG) was subsequently purified by DEAE ion-exchange chromatography.¹² Standard Typhi Vi antigen and rabbit antiserum (titre 160) to *Citrobacter ballerup* Vi antigen was obtained from the Central Research Institute, Kasauli, India. Normal rabbit serum (NRS) was obtained from adult male rabbits.

Co-agglutination test

The co-agglutination test was performed by the method of Rockhill *et al.*⁷ Briefly, the Cowan I strain of *Staphylococcus aureus* (NCTC 8530) was first stabilised. In this method, harvested *Staph. aureus* cells were washed three times with PBS, suspended in formaldehyde 0.5% in PBS for 3 h, washed three times with PBS and then made up to a final suspension of 10% in PBS. The suspension was then heated at 80°C for 1 h with constant stirring, washed three times in PBS and stored as a stabilised suspension 10% in PBS at 4°C until coupled to the antiserum. One ml of this suspension was mixed with 0.2 ml each of *C. ballerup* Vi antiserum, anti-BP IgG and NRS. Each preparation was diluted 1 in 10 before use. The final diluted preparations were named as Vi-coagg, BP-coagg and NRS-coagg, respectively. Sera were tested with all three reagents simultaneously by mixing 50 µl of each

reagent separately with 50 µl of serum and reading the results after 1–2 min. Tests showing agglutination of NRS-coagg reagent with serum were considered to be uninterpretable.⁷

Latex agglutination test

A 1% suspension of latex particles (diameter 0.778 µm; Sigma) was sensitised in 0.1 M glycine–0.15 M NaCl buffer (pH 8.2) with equal volumes of *C. ballerup* Vi antiserum, anti-BP IgG or NRS.¹³ The latex suspensions and antisera were mixed continuously at room temperature for 2 h, after which bovine serum albumin (Sigma) 1% was added and the incubation was continued for 1 h. The latex particles were then washed (10000 g for 10 min) once in 0.1 M glycine–0.15 M NaCl, pH 8.2, buffer and finally suspended as a 1% solution in the same buffer containing bovine serum albumin 1% and sodium azide 0.02%. Latex reagents were denoted as Vi-LA, BP-LA and NRS-LA, respectively. Sera were tested by mixing 30 µl of serum with 10 µl of each latex reagent on a slide for 3–5 min on a mechanical rotator. The results were read visually. Tests showing agglutination of NRS-LA reagent with serum were considered to be uninterpretable.

Control agglutination tests

Protein antigens prepared as described from enteric bacteria other than *Salmonella* serotype Typhi were tested in 10-fold dilutions from 1 ng/ml to 1 mg/ml against all co-agglutination and latex reagents.

Statistical analysis

Statistical analysis was by calculating the following indices:⁴ sensitivity = $[a/(a+c)] \times 100$; specificity = $[d/(b+d)] \times 100$; positive predictive value = $[a/(a+b)] \times 100$; and negative predictive value = $[d/(c+d)] \times 100$; where a is the number of true positive samples, b is the number of false positive samples, c is the number of false negative samples and d is the number of true negative samples.

Results

The results of the co-agglutination and latex agglutination tests in the various patient groups are shown in table I. The latex agglutination test for detection of Vi antigen gave more positive results than any other test for group A patients (96.7%) while latex-based tests for the detection of Vi and BP antigens were both positive in 90% of group B patients. One of the five *E. coli* septicaemia cases (group D) gave a false positive reaction for Vi and BP antigens. There were no false positive reactions in group E. With the co-agglutination test, uninterpretable results were obtained with two sera in group

Table I. Results of co-agglutination and latex agglutination tests

Group	Number of patients	Number (%) of sera with positive reactions					
		Vi-coagg	BP-coagg	NRS-coagg	Vi-LA	BP-LA	NRS-LA
A	30	26 (86.6)	27 (90)	2	29 (96.7)	28 (93.3)	0
B	30	24 (80)	25 (83.3)	3	27 (90)	27 (90)	1
C	30	9 (30)	10 (33)	4	6 (20)	7 (23.3)	1
D	15	1 (6.7)*	1 (6.7)*	0	0	1 (6.7)*	0
E	50	0	0	0	0	0	0

*False positive in one case of *E. coli* septicaemia.

Table II. Statistical analysis of the results of co-agglutination and latex agglutination tests

Test	True positive	False positive	False negative	True negative	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Vi-coagg	50	1	10	64	83.3	98.5	98.0	86.5
Vi-LA	56	—	4	65	93.3	100	100	94.2
BP-coagg	52	1	8	64	86.7	98.5	98.1	88.9
BP-LA	55	1	5	64	91.7	98.5	98.2	92.8

A, three in group B and four in group C. With the latex agglutination test, one serum sample in each of groups B and C produced uninterpretable results.

For the purposes of statistical calculations, groups A and B were considered "true positive" and groups D and E as "true negative". The uninterpretable results were considered as negatives. Of all the tests (table II), latex agglutination for detection of Vi antigen showed the greatest sensitivity (93.3%), specificity (100%), positive predictive value (100%) and negative predictive value (94.2%). The minimum amount of Typhi BP antigen detected by both the co-agglutination and latex agglutination tests was 10 µg/ml. The Vi co-agglutination test and Vi latex agglutination tests could detect 1 µg/ml and 100 ng/ml of Typhi Vi antigen, respectively. Cross-reactivity was observed only with Typhimurium protein antigen (1 mg/ml) which showed agglutination in both the BP co-agglutination and latex agglutination tests.

Discussion

Typhoid fever remains a major public health problem in developing countries and continues to be endemic in many areas. Detection of Typhi antigens in patients' body fluids^{4,5,7,8} has advantages over blood culture and the Widal test, in that the results can be obtained more rapidly, there is no requirement for paired sera and the results are not affected by prior antibiotic therapy.

In the present study, the isolation rate of *Salmonella* serotype Typhi from blood cultures was low with only 30 (50%) of 60 patients with clinical and laboratory evidence of typhoid fever yielding positive cultures. This low isolation rate may be due to prior anti-

bacterial therapy. A lower isolation rate (25.5%) has been reported by Mukherjee *et al.*¹³ In the present work the Vi-LA test detected 96.7% of blood culture-positive patients and 90% of blood culture-negative patients with positive Widal tests. Rapid diagnostic methods for Typhi antigen detection have been performed by many workers.^{6,7,8,13} Rockhill *et al.*⁷ detected Typhi D, Vi and d antigen in the urine of 59 (97%) of 61 bacteriologically proven typhoid fever patients by slide agglutination. Similarly, John *et al.*⁶ reported that 97% of blood culture-positive and 85% of blood culture-negative patients with clinically diagnosed typhoid fever gave positive results in a serum co-agglutination test for the detection of Typhi protein antigen. In a similar study by Mukherjee *et al.*,¹³ a serum co-agglutination test for the detection of Typhi O, H and Vi antigen showed positive results in 96.4% of blood culture-positive patients and 62% of blood culture-negative patients with suspected typhoid fever. In contrast, a very low percentage (34%) of positive results was observed by Taylor *et al.*⁸ with a slide co-agglutination test for the detection of Vi antigen in serum.

A further feature of the present study was that the co-agglutination and latex agglutination tests could detect the presence of Vi and BP antigen in c. 30% and 20% of cases of PUO, respectively. These cases of PUO would have otherwise remained undiagnosed, although for confirmation of typhoid fever in such cases further evaluation of the specificity of these tests will be necessary.

Uninterpretable results obtained with the co-agglutination and latex agglutination tests accounted for 5.5% and 1.2%, respectively, of all specimens tested. These percentages of uninterpretable results are low compared with those of Taylor *et al.*⁸ who observed uninterpretable results in 21% of cases studied.

In developing countries, where there is incomplete notification of infectious diseases and easy availability of antibiotics, patients with enteric fever often report to hospital either after self-medication or administration of antibiotics by private practitioners. In such cases, diagnosis by blood culture is often not confirmed, but detection of soluble antigen by Vi latex agglutination test would still be possible. The Vi latex agglutination test, being the most sensitive (93.3%) and specific (100%), would be useful in primary health centres, as the test does not require a high degree of technical expertise, it is cheap to perform and the results are obtained within a few hours after collection of blood.

Although occasional non-specific reactions or uninterpretable results may limit its use, the Vi latex agglutination test is potentially suitable for the rapid detection of Typhi Vi antigen in patients' sera. However, these results should be further confirmed by conventional methods. In the present study, the number of patients with a proven diagnosis other than typhoid fever was relatively small and since there was one false positive among these 15 patients in several of the tests, it is important to extend this group and thus further validate the specificity of the Vi latex agglutination test.

This work was supported by the Council of Scientific and Industrial Research through its Senior Research Fellowship grant.

References

1. Gilman RH, Termini M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet* 1975; **1**: 1211-1213.
2. Levine MM, Grados O, Gilman RH, Woodward WE, Solis-Plaza R, Waldman W. Diagnostic value of the Widal test in areas endemic for typhoid fever. *Am J Trop Med Hyg* 1978; **27**: 795-800.
3. Abraham G, Teklu B, Gedebu M, Selassie GH, Azene G. Diagnostic value of the Widal test. *Trop Geogr Med* 1981; **33**: 329-333.
4. Appassakij H, Bunchuin N, Sarasombath S *et al.* Enzyme-linked immunosorbent assay for detection of *Salmonella typhi* protein antigen. *J Clin Microbiol* 1987; **25**: 273-277.
5. Gupta AK, Rao KM. Simultaneous detection of *Salmonella typhi* antigen and antibody in serum by counter-immunoelectrophoresis for an early and rapid diagnosis of typhoid fever. *J Immunol Methods* 1979; **30**: 349-353.
6. John TJ, Sivadasan K, Kurien B. Evaluation of passive bacterial agglutination for the diagnosis of typhoid fever. *J Clin Microbiol* 1984; **20**: 751-753.
7. Rockhill RC, Rumans LW, Lesmana M, Dennis DT. Detection of *Salmonella typhi* D, Vi, and d antigens by slide coagglutination, in urine from patients with typhoid fever. *J Clin Microbiol* 1980; **11**: 213-216.
8. Taylor DN, Harris JR, Barrett TJ *et al.* Detection of urinary Vi antigen as a diagnostic test for typhoid fever. *J Clin Microbiol* 1983; **18**: 872-876.
9. Barber C, Vladoianu IR, Dimache G. Contributions to the study of *Salmonella*. Immunological specificity of proteins separated from *Salmonella typhi*. *Immunology* 1966; **11**: 287-296.
10. Hartree EF. Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* 1972; **48**: 422-427.
11. Ekpo P, Sarasombath S, Banchuin N, Sirisinha S. Monoclonal antibodies to 52-Kilodalton protein of *Salmonella typhi*. *J Clin Microbiol* 1990; **28**: 1818-1821.
12. Williams CA, Chase MW. Methods in immunology and immunochemistry, vol II. Physical and chemical methods. New York, Academic Press.
13. Mukherjee C, Malik A, Khan HM, Malik A. Rapid diagnosis of typhoid fever by co-agglutination in an Indian hospital. *J Med Microbiol* 1993; **39**: 74-77.