Concurrent Evaluation of Widal and Counter-immunoelectrophoresis Tests for Enteric Fever*

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SUMMARY **In order to overcome the problems of sensitivity and specificity related to serological tests for typhoid and paratyphoid fevers, we employed antigens prepared from a local strain and established locally significant titres for Widal (TO) (1:80) and counter-immunoelectrophoresis (CIE) (1:4) tests. This was done by testing sera from a large number of healthy individuals (1,153) and by determining the value of carrying out both tests as a method for reducing the rate of false positivity among 20 patients with bacteriologically proven cases of enteric fever and 76 patients infected with other agents. Thirteen out of 13 typhoid fever cases (100%) and five out of seven paratyphoid fever cases (71%) were confirmed by TO based on antibody levels reaching the locally determined significant titre of 1:80 in either or both the acute and convalescent phase sera and/or a four-fold or two-step rise of titre.**

Using the same criteria for the CIE test, 12 out of the 13 typhoid fever cases (92%) and six out of the seven paratyphoid fever cases (86%) could be confirmed. For individuals infected with other agents, the false positive rates for either test were around 5 per cent. However, none of the patients had positive results in both tests. Thus, while CIE alone appears to be less sensitive than TO, when tested concurrently with TO, it can help to exclude false positive cases.

ASIAN PACIFIC J ALLERG IMMUN 1984; 2 : 23-26.

The definitive diagnosis of enteric fever, which is still a prevalent infection in Thailand and many other tropical countries, depends on the isolation of *Salmonellae* from blood, faeces, urine, bone marrow or other body fluids. In areas where the disease is endemic, there often are no facilities for making bacteriological tests and where there are facilities, the cost of the tests in prohibitive. Diagnosis has depended upon the Widal test, a test widely used for nearly a century. However, its value has been questioned particularly with regard to sensitivity and specificity.[1-5] Many of the problems of interpretation can be attributed to variations in antigens and procedures employed, the timing of serum collection and possible effect of antibiotic treatment, and the extent of endemicity or immunisation among the population concerned. Proper control of these variables could improve the value of the test[6,7] but is often difficult to accomplish. In practical terms, it is important to establish a baseline antibody level for the population, particularly in endemic areas, such that a valid cutoff point, i.e., significant titre for that particular population, can be defined. In addition, it is of value to use a bacterial strain of local origin or one that covers the commonly found variety in the area and to employ an easily standardised procedure. In the meantime, attempts have also been made to devise new and hopefully improved tests. Among these new tests is CIE which has provided promising results.[8-10]

The purpose of the present study is two-fold : to compare the usefulness of the CIE and Widal tests, and to explore the value of using both tests concurrently in order to exclude false positivity. In doing so, the level of significant titre for each test was first establised for a large number of subjects, then the value of the tests in bacteriologically proven cases of typhoid and paratyphoid fever as well as patients infected with other agents was assessed.

MATERIALS AND METHODS

Serum

Sera were obtained form 1,153 healthy subjects, 13 patients who had *S. typhi* and seven who had *S.*

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Supported by a Rachadapiseksompoj-China Medical Board Grant.

paratyphi A on haemoculture as well as 76 patients with other infectious diseases. The adult patients with enteric fever were admitted to Chulalongkorn Hospital during a period of 18 months. The time of admission was mostly during the second week following the onset of enteric fever. Sera of this group were obtained on the day of admission to provide the first set of specimens and between one to two weeks later as the second set. The 76 patients with other infectious diseases had urinary tract infections, bacterial pneumonia and viral infections.

Widal Test[11]

Salmonella typhi O (TO) antigen prepared from a local strain of *S. typhi* using the procedure as described[12] was used throughout the study. This bacterial strain contains no detectable "Vi" antigen. In performing the test, an equal volume of antigen was added to each tube containing a two-fold serial dilution of the serum starting with a 1:10 dilution; it was mixed well and incubated at 37°C overnight before the agglutination titre was read and recorded.

Counter-immunoelectrophoresis (CIE) test

CIE was carried out as descibed,[8] employing a veronal buffer extract of acetone-dried bacteria of the same strain used in the Widal test described in this study. The antigen contains O-specific side-chains of lipo-polysaccharide and surface protein antigen.[8] The results were read soon after the running time of 90 minutes. When undiluted (1:1), sera were positive; the antibody titre was estimated by using a serial two-fold dilution procedure starting from 1:2 onward.

RESULTS

The results of the Widal test and the CIE test on sera from the various groups of subjects are shown in Table 1. The positive rate was based on a cutoff point at a reciprocal titre of 80 or above for Widal and 4 or above for CIE and/or a four-fold rise in titre from the acute to convalescent phase of the specimens. These results indicate that both tests are highly sensitive for typhoid but less sensitive for paratyphoid. However, the false positive rate of around 5 per cent among patients with other infections was relatively high.

Titres of individual sera from patients with typhoid and paratyphoid are shown in Table 2 and Table 3 respectively. These results suggest that the titres of the Widal test were already quite high on admission (second week after onset) and thus a significant rise in titre could be seen only among those with low initial titres. The general

Table 1 Positive rates of Widal and CIE tests among various groups of subjects.

Group	Positive/No. tested		Positive rate	
	Widal (reciprocal titre > 80)		CIE (reciprocal titre > 4)	
	1st serum	2nd serum*	1st serum	2nd serum
Control	20/945 (2.1%)	N.T.	2/208 (1.0%)	N.T.
Typhoid	10/13 (76.9%)	13/13 (100%)	7/13 (53.8%)	12/13 (92.3%)
Paratyphoid	4/7 (57.1%)	5/7 (71.4%)	4/7 (57.1%)	6/7 (85.7%)
Others	4/76 (5.3%)	N.T.	4/76 (5.3%)	N.T.

*Positive rate includes those with four-fold rise in titre.
N.T. = Not tested

Table 2 Reciprocal antibody titres of Widal (TO) and CIE tests in 13 typhoid patients.

Patient No.	Widal TO		CIE	
	First specimen	Second specimen	First specimen	Second specimen
1	160	160	1	32
2	160	160	<1	8
3	160	160	8	4
4	320	160	4	8
5	160	320	8	64
6	640	640	32	32
7	80	160	1	4
8	20	160	8	32
9	160	640	4	8
10	160	320	2	4
11	40	80	8	32
12	80	320	2	2
13	40	160	< 1	4

Table 3 Reciprocal antibody titres of Widal (TO) and CIE tests in 7 paratyphoid patients.

Patient No.	Widal TO		CIE	
	First specimen	Second specimen	First specimen	Second specimen
1	40	320	<1	4
2	<20	<20	<1	2
3	80	40	4	<1
4	80	80	4	4
5	160	640	8	32
6	160	160	4	8
7	<20	<20	1	1

trend with CIE was the same except that a significant rise of titre could be observed more frequently.

DISCUSSION

A serological diagnosis of enteric fever depends on two criteria, either a four-fold or two-step rise in antibody titres or titre reaching the diagnostic level on a single specimen taken during the first two to three weeks following the onset of illness. The diagnostic or significant level is usually defined as the serological titre that can be found in not more than 5 per cent of the normal population.[13] Based on these criteria, the diagnostic level of our Widal test was 1 to 80 and the CIE test was 1 in 4 as these levels were found in 2.1 per cent and 1 per cent respectively of the normal population (Table 1). This baseline level of the Widal test is in agreement with a study carried out in Sri Lanka[6] and is one dilution lower than a test in Malaysia,[7] both of which countries are also in the endemic area. The use of the Widal test on typhoid patients showed significant titre in 10 out of the 13 cases (76%) for the first specimen and 13 out of 13 (100%) for the second specimen. For paratyphoid, the sensitivity was somewhat lower (57% and 71% respectively). This study clearly shows that the Widal TO test is still useful in diagnosing typhoid fever when a baseline is properly established and suitable reagents and procedures are employed and particularly when second specimens are available.

The sensitivity of the Widal test to typhoid was similar to the 76-94.3 per cent rates reported by others,[6,7,14-16] except for those reported at rates as low as 59 per cent.[17] One of the causes of low sensitivity is likely to be the unsuitability of the antigen preparation as far as the bacterial strain used and perhaps also the shelf-life etc, of the preparation are concerned. The relatively high sensitivity, at least with the Widal test reported here, could be due in part to the use of a local bacterial strain. However, this claim needs to be further substantiated. The sensitivity of the CIE test, though somewhat lower than the Widal test, is respectable. With further refinement (perhaps an improvement in the preparation of the antigen and optimisation of the electrophoretic process), the sensitivity of CIE for typhoid could be raised further. Nonetheless, the value of CIE at present (when carried out along with the Widal test) in excluding false positive cases cannot be overlooked. It is noteworthy that among the false positive cases none was positive in both of the tests.

Another factor affecting the sensitivity of the tests involved the timing of serum collection. While the admission titres with Widal test were mostly high (and thus a rising titre was not observed), antibodies detected by the CIE test appeared to rise later and a rising titre was observed much more frequently. Since enteric fever is endemic in our country, the antibody titre may be expected to rise early in the Widal test as reported by others.[4,6,7] Most of our patients came to the hospital during the second week with relatively elevated admission titres. Therefore, it is not surprising that a further four-fold rise was not often observed (four out of 13 cases). By contrast, the admission titres measured by CIE were relatively low and a four-fold rise was more frequently observed (seven out of 13 cases). It is likely that the two tests preferentially detect antibodies of differential classes. While it is well recognised that IgM is more efficient than IgG in agglutination -the basis of Widal- IgG may be equally or more efficient in CIE.

The follow-up studies in the present report are limited and not sufficient to define the decline of antibodies by either test.

In cases of paratyphoid, the sensitivity of the CIE test was 57 and 86 per cent with single and double specimens respectively. The CIE test in cases of paratyphoid showed better sensitivity than the Widal test, but that sensitivity was not sufficiently great to preclude the need for devising other methods for determining *Salmonella* antibodies. When compared with the results of Tsang and Chau,[8] who employed similar antigens, methods and subjects and reported a sensitivity level of 96 and 98 per cent when read immediately and after staining respectively, the sensitivity reported in the present study is somewhat lower. The figures again indicate the need for standardisation of both the bacterial strain and the method used. We chose veronal buffer extract antigen for our CIE method because it is easy to prepare and

Tsang and Chau[8] had shown it to be more sensitive than ultrasonic lysate and Barber antigen when the results are read soon after the experiment. This antigen was shown to have a cross-reaction with *E. coli* antigen when *S. typhi* 0 strain NCTC 5753 was used.[8] Hernandez-Velarde *et al*[18] also reported on CIE with a different antigen extraction procedure. The antigen employed contained lipo-polysaccharide obtained by phenol and water extraction and the results in typhoid tests showed reciprocal titres of 8-32, similar to ours which varied from 4 to 64. The antigen used by Jad *et al*[10] was a soluble acid hydrolysate which showed comparable results in undiluted sera with the Widal test. Gupta and Rao[9] also reported that one of 24 acute phase sera gave positive results whereas all 13 convalescent phase sera were positive in the CIE test.

The false reactivity rate among patients with other infectious diseases was about 5 per cent in each of the two tests. False positive rates for the Widal test reported varies between 1 and 3 per cent;[6,7] for the CIE test, between 0 and 10 per cent.[8] The slight difference in the occurrence of false positivity may be due to the factors already described and also to the groups of subjects studied. Our subjects may have included a higher proportion of people infected with bacteria which cross-react with *S. typhi*, such as the Gram-negative bacilli found in urinary tract infections. The specificity of the Widal test could be estimated to be 95-98 per cent and that of the CIE test to be 95-99 per cent based on the findings in cases which showed significant titre levels in the normal controls and other infectious-disease groups and a false positive rate that could be reduced when two independent tests were performed.

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