

Full length Research Paper

Value of a single serum widal agglutination test in diagnosis of paratyphoid fever A

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The diagnostic value of a single-serum Widal agglutination test (WAT) for patients with paratyphoid fever A (PA) was evaluated. 760 PA cases with a blood culture positive for *Salmonella enterica* subsp. *enterica* serovar Paratyphi A (SPA), 920 febrile patients confirmed by a blood culture negative for microbes, and 63 healthy personnel were objects of study. Adopted WAT to examine O (somatic), H (flagellum), A (flagellum) agglutinins antibodies of SPA in sera of 3 groups, obtained O, H, A agglutinins distributions and geometric mean titers in each group. Evaluated and analyzed the diagnostic sensitivity, specificity, predictive value, and probability ratio for the indicated patients of the WAT at different cutoff values. The O, H, A-agglutinin titers were ≥ 160 in 53%, 40%, and 51% of the blood culture positive PA cases, respectively. The O-agglutinin titers of ≥ 160 were found in 20% of the febrile controls and 3.2% of the healthy personnel, the corresponding values for H agglutinins were 17% and 4.8%, the values for A agglutinins were 16% and 1.6%, respectively. The single serum WAT could be of use for diagnosis of PA in patients who have clinical PA but are culture negative or in regions where bacterial culturing facilities are not available.

Keywords: Paratyphoid Fever, Blood Culture, Serum, Widal Agglutination Test

INTRODUCTION

Paratyphoid fever A (PA), caused by *Salmonella enterica* subsp. *enterica* serovar Paratyphi A (SPA), has been a major public health problem in Hongta district (a 1004-km² area with 399,800 registered inhabitants) of Yuxi City, Yunnan Province, China, since 2000 (the incidence of enteric fever was 1 to 8 cases per 100,000 population per year during 1991 to 1999). Causing an estimated 1,000 new infections confirmed by blood culture positive for SPA each year in Hongta district alone, the incidence of PA was high up to 900 cases per 100,000 population per year in a few communities. PA has been endemic in Hongta district, the resulting Widal agglutination test (WAT) result may lack sensitivity and/or specificity (Parry CM et

al., 1999; Parry CM et al., 2004). We have evaluated the value of a single serum WAT result for the diagnosis of PA.

The signs and symptoms of PA show no specificity, this kind of disease is hard to be diagnosed accurately on clinical grounds, Although a definitive diagnosis can be made by isolation of SPA from a clinical specimen like blood, bone marrow, urine, or stool, or by demonstrating the rising titers of O (somatic), H (flagellum), and A (flagellum) agglutinins antibodies in paired serum samples by the Widal agglutination test (WAT), it is the common case that it is hard to get microbiological culturing facilities and paired serum samples (Olsen et al., 2004; Parry CM et al., 2004). The WAT has been in use for more than a century as an aid in the diagnosis of typhoid and paratyphoid fever in the original format, there is no consensus concerning diagnostic criteria for interpreting the test, the test required acute- and convalescent-phase

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serum samples taken approximately 10-14 days apart, with a fourfold rise in serum antibody titer between the two samples being diagnostic of PA (Grunbaum, 1896; House et al., 2005; Olopoenia et al., 2000). Serological diagnosis for PA relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart. In typhoid and paratyphoid fever cases, however, such a rise is not always demonstrable, the situation may occur because the acute-phase sample was obtained late in the natural history of the disease, because of the high levels of background antibodies in an endemic region, or because in some individuals the antibody response is blunted by the early administration of an antibiotic (Harries et al., 1995). Furthermore, the patient management cannot wait for results obtained with a convalescent-phase sample. For practical purposes, a treatment decision must be made on the basis of the results obtained with a single acute-phase sample.

MATERIALS AND METHODS

Study groups

Group 1: 760 PA patients admitted to municipal center for diseases control and prevention and municipal hospital of Yuxi City between July 2001 and May 2007 were confirmed by blood culture positive for SPA. Information concerning the duration of illness before admission was available for all these patients. The duration of illness ranged from 1 day to 27 days, the percentile 50 (P_{50}) and P_{95} was 8.4 d and 14 d, respectively, when the blood samples were collected. The cases (group 1) were subdivided into the two subgroups of patients according to the duration of illness. The number of patients with the duration of illness ≤ 7 d (short illness history) is 290; the number of patients with the duration of illness > 7 d (long illness history) is 470. Group 2: 500 febrile patients with negative blood culture. Group 3: 63 healthy food and drink handlers.

Experimental materials

Standard blood culture bottles containing brain heart infusion broth are supplied by Biomerieux in France. Culture media including Columbia agar, nutrient agar and Salmonella-Shigella agar are supplied by Oxoid in England. Salmonella O2- and Ha- specific antisera as well as bacterial suspensions of *Salmonella enterica* subsp. *enterica* serovar Typhi and SPA produced by biological products institute of Ministry of Public Health, Lanzhou, China.

Laboratory analysis

Blood culture: 10ml venous blood (3 to 5ml from children under 12 years old) was drawn aseptically from each patient Group 1, 2 and inoculated into 50ml blood culture bottle. The blood culture bottle was then incubated at 37°C for 2 to 7 days (Wain et al., 1998). All bottles were examined daily, if a bottle showed visible signs of growth, the broth was subcultured onto sheep blood agar. Bacterial isolates were identified by agglutination with Salmonella O2- and Ha-specific antisera and standard biochemical tests (Biomerieux Automatic Microorganism Identification System VITEK 32 or API 20E, France). WAT: Blood samples from all study groups (Group 1, 2, 3) were centrifuged, and the serum specimens were divided into aliquots and stored at -20°C. In order to minimize the degradation of the antibodies in the sera, the sera were frozen immediately and remained frozen until the time of testing. The WAT involves the use of bacterial suspensions of *Salmonella enterica* subsp. *enterica* serovar Typhi and SPA, treated to retain only the "O" or "H" or "A" antigens, the antigens are employed to detect corresponding antibodies in the serum of a person. Serum was serially diluted, starting at 1/10, in physiological saline water and then further diluted 1/2 in suspensions containing "O", "H" and "A" antigens, separately. Tubes containing "O" or "H" or "A" antigens and sera were incubated at 37°C for up to 20 hours, and examined for visible agglutination (Olopoenia et al., 2000). Appropriate positive and negative control sera were included.

Evaluation on WAT for the diagnosis of PA

Take the blood culture technique as the golden criterion. Sensitivity (true-positive rate) was defined as the probability that the WAT result would be positive when blood culture confirmed that SPA was present (group 1). Specificity (true-negative rate) was the probability that the WAT result would be negative when SPA was not present (groups 2 and 3). The positive predictive value was the probability that SPA was present when the test was positive, and the negative predictive value was the probability that SPA was not present when the test was negative. Although the sensitivity and specificity were not affected by the prevalence of SPA, the predictive values depended strongly on the prevalence (House et al., 2001). According to the following table for evaluation of diagnostic test (table 1), evaluate the value of WAT using the following indexes: sensitivity = $a/(a+c)$; specificity = $d/(b+d)$; positive predictive value = $a/(a+b)$; negative predictive value = $d/(c+d)$; positive probability ratio = $[a/(a+c)]/[b/(b+d)]$; negative probability ratio =

Table 1: Evaluation of diagnostic test

Diagnostic test	Golden criterion		Total
	Patients	normal persons	
+ positive	a true positive	b false positive	a+b
- negative	c false negative	d true negative	c+d
total	a+c	b+d	n

Table 2: O, H, A agglutinins of the three crowds^a

Group (NO. of persons)	NO. of persons in subgroups	S. serotype paratyphi antigen	NO.(%) of sera showing the following reciprocal antibody titer						Geometric mean titers of antibodies
			≤20	40	80	160	320	640	
1(760)	290(the duration of illness≤7d)	O	60(21)	30(10)	110(38)	40(14)	50(17)	0	78.0
		H	100(34)	60(21)	60(21)	60(21)	10(3)	0	52.0
		A	80(28)	70(24)	60(21)	30(10)	50(17)	0	63.0
	470(the duration of illness>7d)	O	40(9)	60(13)	60(13)	140(30)	160(33)	10(2)	134
		H	40(9)	50(11)	140(30)	180(38)	60(12)	0	103
		A	30(6)	10(2)	120(26)	120(26)	180(38)	10(2)	154
2(500)		O	140(28)	160(32)	130(26)	40(8)	30(6)	0	50.0
		H	180(36)	170(34)	80(16)	40(8)	30(6)	0	44.1
		A	210(42)	80(16)	160(32)	40(8)	10(2)	0	43.5
3(63)		O	29(46)	12(19)	20(32)	2(3)	0	0	37.9
		H	30(48)	17(27)	13(21)	3(5)	0	0	35.4
		A	48(76)	11(17)	3(5)	1(2)	0	0	24.9

a For "O" or "H" or "A" entries, the person's highest titer is shown.

$$[c/(a+c)]/[d/(b+d)].$$

RESULTS

The WAT titers for the three groups

The distribution and geometric mean titers of O, H, A agglutinins for the three groups are shown in table 2. Seen from table 2, the geometric mean titers of O, H, A antibodies in sera of patients with the duration of illness >7 days are nearly twice as much as that of patients with the duration of illness ≤7 days; the O, H, A agglutinins titers which were ≥160 in the former took up 65%, 50% and 66% respectively. The relevant O, H, A agglutinins titers in the latter took up 31%, 24% and 27%, separately. The geometric mean titers of O, H, A antibodies of patients with the duration of illness >7 days are triple and even six times as much as those of healthy food and drink handlers. The geometric mean titers of O, H, A antibodies of febrile patients with blood culture negative in group 2 are higher than those in group 3. The O agglutinins were

present at a titer of ≥160 in 14% of group 2 and were present at a titer of ≥160 in 3.0% in group 3. Meanwhile, the H agglutinins titers in group 2 and 3 were 14% and 5% respectively, the A agglutinins titers in group 2 and 3 were 14% and 2% respectively. The O-agglutinin titers were ≥160 in 53% of PA cases with blood culture positive, the H and A agglutinin titers were ≥160 in 40% and 51%, respectively. The O-agglutinin titers of ≥160 were found in 20% of the febrile controls and 3.2% of the healthy personnel, the corresponding values for H agglutinins were 17% and 4.8%; the values for A agglutinins were 16% and 1.6%, respectively.

The evaluation of diagnostic value of WAT

Divided group 1(G) into two subgroups (G1, G2), G1 represents the patients with the duration of illness ≤7d, G2 represents the patients with the duration of illness >7d. Group 2 was the controlled group. In accordance with different values of O, H, A, calculated the indications including sensitivity, specificity, positive and negative

Table 3: Value of WAT in diagnosis of paratyphoid fever A

Antigen	Agglutinin titer	Sensitivity			Specificity	Predictive value			Probability ratio								
		G	G ₁	G ₂		Positive			Negative			Positive			Negative		
						G	G ₁	G ₂	G	G ₁	G ₂	G	G ₁	G ₂	G	G ₁	G ₂
O	≥40	0.87	0.79	0.91	0.22	0.48	0.24	0.37	0.67	0.77	0.83	1.1	1.0	1.2	0.59	0.95	0.37
	≥80	0.76	0.69	0.79	0.48	0.50	0.29	0.44	0.70	0.83	0.81	1.4	1.3	1.5	0.52	0.65	0.44
	≥160	0.53	0.31	0.66	0.80	0.69	0.33	0.63	0.67	0.79	0.81	2.7	1.6	3.3	0.49	0.86	0.43
H	≥40	0.82	0.66	0.91	0.27	0.45	0.22	0.39	0.64	0.71	0.86	1.1	0.90	1.2	0.678	1.3	0.31
	≥80	0.67	0.45	0.81	0.57	0.56	0.25	0.49	0.68	0.76	0.85	1.6	1.0	1.9	0.58	0.96	0.33
	≥160	0.41	0.24	0.51	0.83	0.66	0.30	0.60	0.63	0.78	0.77	2.4	1.4	3.0	0.71	0.92	0.59
A	≥40	0.86	0.72	0.94	0.34	0.52	0.26	0.42	0.74	0.79	0.91	1.3	1.1	1.4	0.41	0.82	0.19
	≥80	0.75	0.72	0.94	0.52	0.56	0.32	0.50	0.72	0.86	0.94	1.6	1.5	2.0	0.48	0.54	0.12
	≥160	0.51	0.28	0.66	0.84	0.72	0.35	0.67	0.74	0.79	0.83	3.2	1.8	4.1	0.43	0.86	0.40
CorHorA	≥80	0.93	0.86	0.97	0.28	0.52	0.27	0.42	0.84	0.87	0.96	1.3	1.2	1.4	0.24	0.50	0.071
CorHorA	≥160	0.64	0.41	0.79	0.68	0.63	0.29	0.56	0.70	0.79	0.86	2.0	1.3	2.5	0.53	0.87	0.31
CorA	O≥160orA≥80	0.78	0.59	0.89	0.54	0.53	0.29	0.50	0.75	0.81	0.91	1.7	1.3	1.9	0.41	0.76	0.20

predictive value, positive and negative probability ratio (table 3). With a cutoff titer of ≥160 for O or H or A agglutinin, the WAT would diagnose correctly 64% of PA cases with the blood culture positive. However, 37% of the positive results would be false positive, and 30% of the negative results would be false negative. With a cutoff titer of ≥160 for A agglutinin, the WAT would diagnose correctly 51% of PA cases with blood culture positive. However, 28% of the positive results would be false positive, and 26% of the negative results would be false negative.

DISCUSSIONS

The specific purpose of this study was to know the law of immune response in patients with PA and to develop local recommendations for the interpretation of WAT results. The serum antibody responses were investigated with individuals from a region of China in which PA is endemic, and their usefulness for the diagnosis of PA was evaluated.

There are various difficulties associated with an evaluation of the WAT. First, the levels of agglutinins detectable in the noninfected populations of different districts vary considerably (Pang et al., 1983; Parry CM et al., 1999; Parry CM et al., 2004; Senewiratne et al., 1977). The variation depends on the degree to which the disease is endemic in each district, a fact which may change over time. It also depends on the level of infection due to other salmonellae with cross-reacting antigens. Second, the choice of a satisfactory gold standard for diagnosis and the selection of an adequate control group. In this study,

we have chosen PA patients with blood cultured-positive as the confirmed cases. However, some patients with PA will be blood culture negative for antibiotic pretreatment is common. The ideal control group would be patients with an illness compatible with PA or typhoid fever and investigated with WAT but who are then found not to have PA or typhoid fever. However, it can be difficult to choose patients who are blood culture negative and definitely don't have PA or typhoid fever.

This study confirms the presence of detectable agglutinins in population without PA or typhoid fever. O agglutinins were present at a titer of ≥160 in 3%, H agglutinins were present at a titer of ≥160 in 5% and A agglutinins were present at a titer of ≥160 in 2% of 63 health drink and food handlers. The levels of agglutinins are similar to those found in other studies (Frimpong et al., 2000; Parry CM et al., 1999; Parry CM et al., 2004; Senewiratne et al., 1977). Salmonellae are divided into distinct serological groups (A through E) on the basis of their somatic O antigens. All groups A and B organisms, such as SPA possess O antigen 12. Thus, infection by any of the groups A and B serotypes can produce antibodies that can react with the O antigen used in the WAT. Also, since most group D serotypes have O antigen 12, cross-reactions producing a false-positive O-antigen titer in the WAT can therefore occur with any of these serotypes (Olopoenia et al., 2000; Reynolds et al., 1966). PA resulted in a positive O-antigen titer of ≥100 in 53% of patients and a positive H-antigen titer of ≥100 in 22% (Parry et al., 1999).

The IgM somatic O antibody appears first and represents the initial serologic response of patients in

acute PA or typhoid fever cases, while the IgG flagella H antibody usually develops more slowly but persists for longer than that of the O antibody (Anon, 1978; Parry et al., 1999). In his study, 71% of patients presenting with ≤ 7 days of fever and with blood culture positive PA had detectable O antibodies at a cutoff titer of 80, 45% had detectable H antibodies at a cutoff titer of 80, and 48% had detectable A antibodies at a cutoff titer of 80. 78% of patients presenting with > 7 days of fever and with blood culture positive PA had detectable O antibodies at a cutoff titer of 80, 80% had detectable H antibodies at a cutoff titer of 80, and 92% had detectable A antibodies at a cutoff titer of 80. Although many patients may have had antibodies at a lower titer, it is well recognized that patients with confirmed PA may have a negative WAT throughout the course of their illness, although the proportion has varied in different reports (Pang et al., 1983; Parry et al., 1999). This lack of antibody response among patients with blood culture positive PA has been attributed to undefined host (the carrier state) or bacterial factors or previous antibiotic treatment (Opolopoenia et al., 2000; Senewiratne et al., 1977). In this study, Some patients were investigated in the first week of the disease, and this early investigation might be thought to have contributed to the proportion of patients with negative results. However, the traditional view that the test becomes positive only in the second week of the illness is not supported by our data (Anon, 1978; Grunbaum, 1896; Schroeder, 1968; Senewiratne et al., 1977). The test was positive (titer to O and/or H and/or A antigen of ≥ 80) in 50% of patients with blood culture positive PA during the first 5 days of their illness. This result may reflect a population immunologically sensitized by regular subclinical exposure to SPA.

False positive Widal test results have been reported for patients with nonenteric fever *Salmonellae* infections, malaria, typhus, immunological disorders, and chronic liver disease (Harries et al., 1995; Pang et al., 1983; Senewiratne et al., 1977). Elevated levels of agglutinins were found in patients with a variety of other bacteremic illnesses, including those caused by other *Salmonella* spp., *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus aureus*, the level of O antibodies in these patients was higher than that of H antibodies (Parry et al., 1999). Sansone et al published a case report where the WAT to O antigen on admission for an unexposed patient was 1:320, with an increase in titer to 1:20,480 by the fourth day. While both blood and urine cultures were negative for *Salmonella enteric* serovar Typhi in this case, a non-typhoidal *Salmonella* spp. was isolated from the stool of this patient which was identified as *Salmonella javiana* (Sansone et al. 1972). It should be stressed that a single WAT has no diagnostic significance unless the sensitivity and specificity of the WAT for the specific laboratory and patient population are known as well as

predictive value.

The predictive value of a diagnostic test depends on the sensitivity and specificity of the test and on the prevalence of the disease in the population being tested. The Sensitivity and positive probability ratio of WAT increased along with the increase of course before drawing blood. The sensitivity and positive probability ratio of group with course $> 7d$ were obviously higher than that of group with course $\leq 7d$ ($p < 0.01$); The performance of the WAT will vary according to the likelihood of PA in the group of patients being investigated. A negative result would have a good predictive value for the absence of disease, but a positive result would have a very low predictive value for PA. The result would be virtually useless for diagnosing PA. The WAT should be restricted to those who have a reasonable probability of having PA. In our city, the disease prevalence was approximately 45% in those investigated population with a blood culture. At a prevalence of 45%, the results of no agglutination for O-, H- and A- antigen titer have a 99% probability of excluding PA. A positive cutoff for an A-antigen titer of ≥ 160 is frequently used in our city. At this cutoff value, 51% of patients with blood culture positive PA would be correctly diagnosed. However, 28% of the positive results would be false positive and 26% of negative results would be false negative. The positive probability ratio in G, G1 and G2 crowds with a positive cutoff value for A-antigen titer of ≥ 160 were 3.2, 1.8 and 4.1, respectively. With a positive cutoff value for an O- or H or A-antigen titer of ≥ 160 , 64% of patients with blood culture positive PA would be correctly diagnosed. 37% of the positive results would be false positive and 30% of negative results would be false negative. The positive probability ratio in G, G1 and G2 crowds were 2.0, 1.3 and 2.5.

CONCLUSION

The role of the WAT had been to increase the index of suspicion for the presence of enteric fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a four-fold rise of antibody titer. Elevated levels of agglutinating O, H, and A antibodies as measured in the WAT can be helpful in making a presumptive diagnosis of PA if interpreted with care. Ideally, the WAT should be run on both acute-and convalescent-phase sera to detect an increase in the agglutination titer. However, to inform treatment decisions before convalescent samples can be obtained, it is common for a single acute-phase serum sample to be run. When the O-or H-or A-agglutinin titre is ≥ 320 , typhoid or paratyphoid fever A can be diagnosed with reasonable confidence. However, a result that appears to be a false positive test compared to a blood culture may in fact be a true positive, a false positive may

be the result of past infection with serotype paratyphi or another non-paratyphoidal *Salmonella* serotype that shares common antigens.

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