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WIDAL TEST AND ITS MEDICAL SIGNIFICANCE IN DIAGNOSIS

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ABSTRACT

Background: Febrile condition is a common disease caused by different pathogens. The most common microorganism that causes it is *Salmonella typhi* and *Salmonella paratyphi*. The single Widal agglutination test rather than blood culture or other cultures is commonly used in diagnose typhoid fever.

Aim of the study: To evaluate the medical significance and value of a single Widal test result in diagnosis febrile typhoid fever patients.

Material and methods: It is a retrospective study on febrile patients to diagnose typhoid fever and 100 patients were enrolled in the study. Blood sample were collected from the patients and tested for qualitative Widal slide agglutination test for confirmation of the disease. This test is used for detection the presence of anti *Salmonella typhi* O and H antibodies, anti *Salmonella paratyphi* A (O and H) antibodies, anti *Salmonella paratyphi* B (O and H) antibodies in the patients' serum.

Results: Antibodies against S typhi O antigen were 72/100 (72%), and to H antigens was 64/100 (64%). Regarding S paratyphi A, the reactive O antigen was 34/100 (34%) and H antigen was 16/100 (16%) while *S paratyphi* O antigen was 23/100 (23%) and H was 14/100 (14%). Among those patients who had positive agglutination reaction for *S typhi* results, 22 (30.55%) had titer of 1:360 for O antigen and 37 (57.81%) had titer of 1:40 of H antigen of *S typhi*. **Conclusions:** Single Widal agglutination test is used as diagnosis of Typhoid fever by interpretation of both O and H antigen and the cut-off titer in Iraq was 160 because it's endemic in this area.

Keywords: Widal, agglutination, typhoid, *Salmonella typhi* and *Salmonella paratyphi*

INTRODUCTION

Widal agglutination test has been widely used in diagnosis of typhoid fever for many years ago (1). However, the test interpretation remains debatable in many areas in the world whether the disease is endemic or not in that area. The principle of this test is agglutination and interpretation of this test depends on agglutination that occurs at different titrations of the serum of the patient. Interpreting of the test is a problem that different cut-offs have been reported from different areas in the world and paired tests are often difficult so single unpaired test has to be used for screening, diagnosis and treatment (2, 3). Thus the treatment decision must be done on the basis of the results obtained with a single test to serum sample (4, 5). So evaluating the result of a single Widal test is necessary for correct interpretation to the diagnosis and treatment of typhoid fever (6,7). There are many choices for the diagnosis of typhoid fever like clinical signs and symptoms, serological test, bacterial culture, blood culture, stool culture, bone marrow culture, antigen detection and DNA amplification (8,9). In many developing countries like Iraq the Widal test is the most widely used test in diagnosis this disease (typhoid fever) because it is cheap, easy to do and requires minimal training and apparatus (10,11).

So this study was carried out to evaluate the medical significance and value of a single Widal test result in diagnosis febrile typhoid fever patients.

PATIENTS AND METHODS

The study was done in Baghdad- private clinical laboratories and Al-Kindy Teaching Hospital from January 2018 to December 2018. It is a retrospective study on febrile patients to diagnose typhoid fever and 100 patients were enrolled in the study. The inclusion criteria were patients examined by their physician for the clinical signs and symptoms of typhoid fever which includes fever more than two days accompanied by other clinical symptoms like splenomegaly in the absence of any other causes of febrile illnesses. The exclusion criteria were febrile patients who received antibiotic for their symptom within one to two weeks before consulting their physician and patients who had febrile illness due to other causes. The study was approved by scientific ethical committee of Al-Kindy College of medicine and Al-Kindy Teaching Hospital. Informed consents were obtained from patients.

Blood sample were collected from the patients and tested for qualitative Widal slide agglutination test for confirmation of the disease using febrile agglutination kit (Agappe diagnostics Switzerland GmbH-Switzerland). This test is used for detection

the presence of anti *Salmonella typhi* O and H antibodies, anti *Salmonella paratyphi* A (O and H) antibodies, anti *Salmonella paratyphi* B (O and H) antibodies in the patients' serum. The test was done using slide agglutination method by adding a drop of *Salmonella typhi* O and H antigens to a drop of serum on a slide and rotated and tilted for one to three minutes and reported as positive or negative. For serums whose slide agglutinations results were positive and weakly positive then titer was determined according to instructions of the kit. An antibody titer to all O antigen of 1:160 and higher were taken as a cut off value to indicate recent infection with *Salmonella*.

Statistical analysis was done using MiniTab software.

RESULTS

One hundred febrile patients' serums were analyzed. Males constituted 70% of them and the rest were females. Their age ranged from 16-63 years (33.67 ± 11.3). Qualitative slide agglutination Widal test was done as a first screening test to detect antibodies against O antigen and H antigens of *S.typhi* and *Salmonella paratyphi*. Antibodies

against *S typhi* O antigen were 72/100 (72%), and to H antigens was 64/100 (64%). Regarding *S paratyphi* A, the reactive O antigen was 34/100 (34%) and H antigen was 16/100 (16%) while *S paratyphi* O antigen was 23/100 (23%) and H was 14/100 (14%) as shown in table-1-.

Semi quantitative Widal agglutination test was done for those patients with positive reaction. The frequency distribution of titration of positive reaction is shown in Table -2-. Among those patients who had positive agglutination reaction for *S typhi* results, 22 (30.55%) had titer of 1:360 for O antigen and 37 (57.81%) had titer of 1:40 of H antigen of *S typhi*. Regarding anti *S paratyphi* A O antigen antibody titer 1:360 was 2(5.88%) while anti H antibody titer 1:40 was 9 (56.25%) and there was no titer of 1:360 H antigen. Other bacteria was *S paratyphi* B and there was no antibody higher titer (1:160 and more) regarding O antigen while H antigen was 10 (71.42%) in 1:40 titer. Antibody titer of 1:160 for O antigen and for H antigens were taken as cut of values to indicate recent typhoid infection (positive titer) because this bacteria is endemic in Iraq.

Table 1: Qualitative slide agglutination Widal test results of febrile patients.

Type of bacterial antigen	Type of reaction			
	Positive		Negative	
	frequency	%	frequency	%
<i>S typhi</i> O antigen	72	72	28	28
<i>S typhi</i> H antigen	64	64	36	36
<i>S paratyphi</i> A O antigen	34	34	66	66
<i>S paratyphi</i> A H antigen	16	16	84	84
<i>S paratyphi</i> B O antigen	23	23	77	77
<i>S Paratyphi</i> B H antigen	14	14	86	86

Table 2: Frequency of semi quantitative febrile agglutination Widal test in febrile patients

Titer	<i>S typhi</i> O antigen		<i>S typhi</i> H antigen	
	Frequency	%	Frequency	%
1:40	10	13.88	37	57.81
1:80	16	22.22	14	21.87
1:160	24	33.33	9	14.06
1:360	22	30.55	4	6.25
Total	72	100	64	100
	<i>S paratyphi</i> A O antigen		<i>S paratyphi</i> A H antigen	
	Frequency	%	Frequency	%
1:40	14	41.17	9	56.25
1:80	15	44.11	7	43.75
1:160	3	8.82	0	0
1:360	2	5.88	0	0
Total	34	100	16	100
	<i>S paratyphi</i> B O antigen		<i>S paratyphi</i> B H antigen	
	Frequency	%	Frequency	%
1:40	11	47.82	10	71.42
1:80	12	52.17	4	28.57
1:160	0	0	0	0
1:360	0	0	0	0
Total	23	100	14	100

DISCUSSION

Febrile illness is a common disease that is caused by many pathogens and *S. typhi* and *S paratyphi* A and B are one of these pathogens. This study showed that Widal test can diagnose *S typhi* in 72% of the febrile patients with a titer of 1:160 in 24 patients (33.33%) and 1:360 in 22 patients (30.55%) followed by *S paratyphi* A and *S paratyphi* B and this was confirmed with clinical presentation and response to treatment. Andualem G. et al .2014 (12) demonstrated that Widal test has a low sensitivity, (71.4%) specificity (68.44%) and positive predictive value (5.7%), but it has good negative predictive value (98.9%) which indicates that negative Widal test result in febrile patients have a good suggestion for the absence of the disease. So a single Widal agglutination test is not a valid diagnostic test in some countries

where other febrile diseases are common like malaria in addition to typhoid fever (13). Thus in Iraq which is like other countries the Widal agglutination test in spite of its limitations in interpretations, it is the most common diagnostic test used in the diagnosis of typhoid fever in association with clinical findings of the patients because of its cheap, easy to perform, requires slight training and no equipment's (14). Paired Widal tests like after treatment or during follow-up are often not easy and possible; so single unpaired test has to be used for screening, diagnosis and treatment that need to apply cut-off to this single Widal test. The cut-off titer in Iraq was 160 because its endemic in this area and most of the normal population had a lower titer like 20 or 40. The interpretation of the Widal test must be done in combination of O and H antigen

because O antibodies indicate recent infection and H antibodies indicate immunity either from previous infection or vaccination like restaurants workers. Other study showed that singular Widal test normal range was 1:20 - 1:80 for *S typhi* O, *S typhi* H, *S paratyphi* AO, *S paratyphi* AH, *S paratyphi* BO, *S paratyphi* BH (15). Further studies are required to determine the cut off values of each area according to its endemicity.

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