

Determination of baseline Widal titre in healthy individuals in Karbala city, South of Iraq

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Objective To determine the cutoff of antibodies directed against *Salmonella enterica* subspecies enterica serotype typhi, paratyphi A, and paratyphi B in normal population in Karbala city, south Iraq.

Methods Widal test was performed for 90 serially diluted serum samples collected from apparently healthy volunteers. A commercial Widal test kit was used. Agglutination results were observed within 1 minute.

Results In the current study, 23.33%, 22.22%, 6.6%, 4.4%, 38.88% and 5.55% of individuals had antibody titre $\geq 1:20$ for *S. enterica* subspecies enterica serotype typhi O, H, *S. enterica* serotype paratyphi AO, AH, *S. enterica* serotype paratyphi BO and BH, respectively.

Conclusion Based on these results, antibody titres of 1:160 for O and H, 1:40 for AO, 1:80 for AH and BH and 1:320 for BO antigens were considered as baseline titre in Karbala.

Keywords *Salmonella enterica*, Widal test, anti-O,H agglutinins

Introduction

Iraq is considered as one of the many endemic countries with typhoid fever.¹ The definitive diagnosis of typhoid fever requires the isolation of *Salmonella enterica* serotype typhi from the blood, feces, urine or other body fluids. In developing countries, facilities for isolation and culture are often not available especially in smaller hospitals, and diagnosis relies upon the clinical features of the disease and the detection of agglutinating antibodies to *S. enterica* serotype typhi by the Widal test, which has been used very extensively in the sero-diagnosis of typhoid fever and, in developing countries it remains the only practical test available. Many studies²⁻⁶ however, have produced data which have cast serious doubts on the value of the Widal test. Classically, a fourfold rise of antibody in paired sera is considered diagnostic of typhoid fever.⁷ However, paired sera are often difficult to obtain and specific chemotherapy has to be instituted on the basis of a single Widal test. Determination of the baseline titre of agglutination of antibodies against salmonella typhi and salmonella paratyphi in healthy individuals in Karbala has not been established before. Thus, the current study concentrates on the determination of cutoff value related to this area.

Materials and Methods

The current study was conducted in the Department of Clinical Laboratory, College of Applied Medical Sciences, Karbala, south Iraq from September to November 2014.

Ninety healthy individuals with different age and sex who reside in various places of Karbala district were enrolled. None of the volunteers had a history of recent vaccination or recorded infection with *Salmonella* or other infectious disease. Mean age of individuals was 25 ± 8.27 years. Number of male volunteers were 60 while female volunteers were 30. The male:female ratio was 2/1. Commercially available Widal test kit (SPINREACT, S.A. Ctra Santa Coloma, Sant Esteve de Bas, Spain) was used. The antigens provided within this kit include antigen suspension of *S. enterica* serotype typhi O, H and serotype paratyphi AO, AH, BO, BH, Brucella and Proteus in glycine buffer, pH 8.2 preservative.

The blood was collected in gel tube containers from volunteers, and left for 15 minutes for clotting, then centrifugation was done for 5 minutes on 6000 RPM. The serum collected after centrifugation was properly labelled for Widal test procedure. The Widal test procedure in this study is slide agglutination method (semi quantitative). We delivered 80, 40, 20, 10 and 5 μL of undiluted serum into separate circles of the slide test. Then, one drop (50 μL) of the antigen was placed on each circle. Disposable stick was used for mixing and spreading of the reactants over the entire area enclosed by the circle. After 1 minute, agglutination was observed by naked eyes.

Statistical Analysis

Statistical analysis was done using SPSS (Statistical Package for the Social Sciences; IBM) version 20. A P value 0.05 was considered significant at a confidence interval of 95% according to chi-square test.

Results

A total of 90 healthy adult volunteers of different age, sex and socioeconomic groups were screened for the agglutination against *S. enterica* subspecies enterica serotypes, typhi, paratyphi A and paratyphi B (Table 1). Sixty were males and 30 were females, male/female ratio was 2/1. Mean age was 24.9 ± 8.27 years.

In the current study, 69 (76.7%), 70 (77.7%), 84 (93.3%), 87 (96.7%), 60 (66.6%) and 85 (94.4%) cases had antibody titres less than 1:20 for O, H, AO, AH, BO and BH, respectively. Whereas, 21 (23.3%) had anti-O agglutinins more than or equal to 1:20. In addition, antibody titre $\geq 1:20$ were seen in

Table 1. Demographic data

	Male	Female	Total
Number	60	30	90
Mean age \pm SD	24.2 \pm 7.7	23.09 \pm 8.55	24.95 \pm 8.27

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Table 2. Frequencies of agglutinins

Agglutinin	N (%) <1:20	N (%) ≥1:20
O agglutinins	69 (76.7)	21 (23.3)
H agglutinins	70 (77.8)	20 (22.2)
AO	84 (93.3)	6 (6.7)
AH	87 (96.7)	3 (3.3)
BO	60 (66.7)	30 (33.3)
BH	85 (94.4)	5 (5.6)

21 (23.3%), 20 (22.2%), 6 (6.7%), 3 (3.3%), 30 (33.3%) and 5 (5.6%) for O, H, AO, AH, BO and BH, respectively (Table 2). Fig. 1 shows the distribution of the positive cases with ≥1:20 titre among the different titres.

The current study revealed that there were significant differences between the presence of O, BO agglutinins and sex, as shown in Table 3.

As shown in Table 4, among 21 cases who had titre ≥1:20 anti-O agglutinins, 15 had titre ≥1:20 anti-H, 5 had titre ≥1:20 anti-AO, 13 had titre ≥1:20 anti-BO, 2 had titre ≥1:20 anti-BH and 4 had titre ≥1:20 anti-AH agglutinins. There were positive correlations between the presence of O agglutinins and H, AO, AH and BO agglutinins.

Cutoff Value

According to the equation (mean ± 2SD) the cutoff values for O and H is 1:160, 1:40 for AO, 1:80 for AH and BH, and 1:320 for BO, are considered as baseline titre in this region (Table 5).

Discussion

Iraq is one of the developing countries where enteric fever is a major public health problem associated with significant morbidity and mortality.^{8,9} Blood culture has remained the gold standard test in diagnosis of typhoid fever, but its utility in early diagnosis is limited and does not exceed 50% even in the best laboratory.¹⁰ The administration of antibiotic therapy before diagnosis is considered to be one of the main reasons for poor isolation rates.¹⁰ Thus, diagnosis of typhoid fever largely relies upon the clinical features and serological tests like Widal.¹⁰ Controversies over the diagnostic utility of Widal could be due to several factors like high endemicity, antigenic cross reactivity with other agents, non-availability of paired sera for the documentation of rising titres, diverse

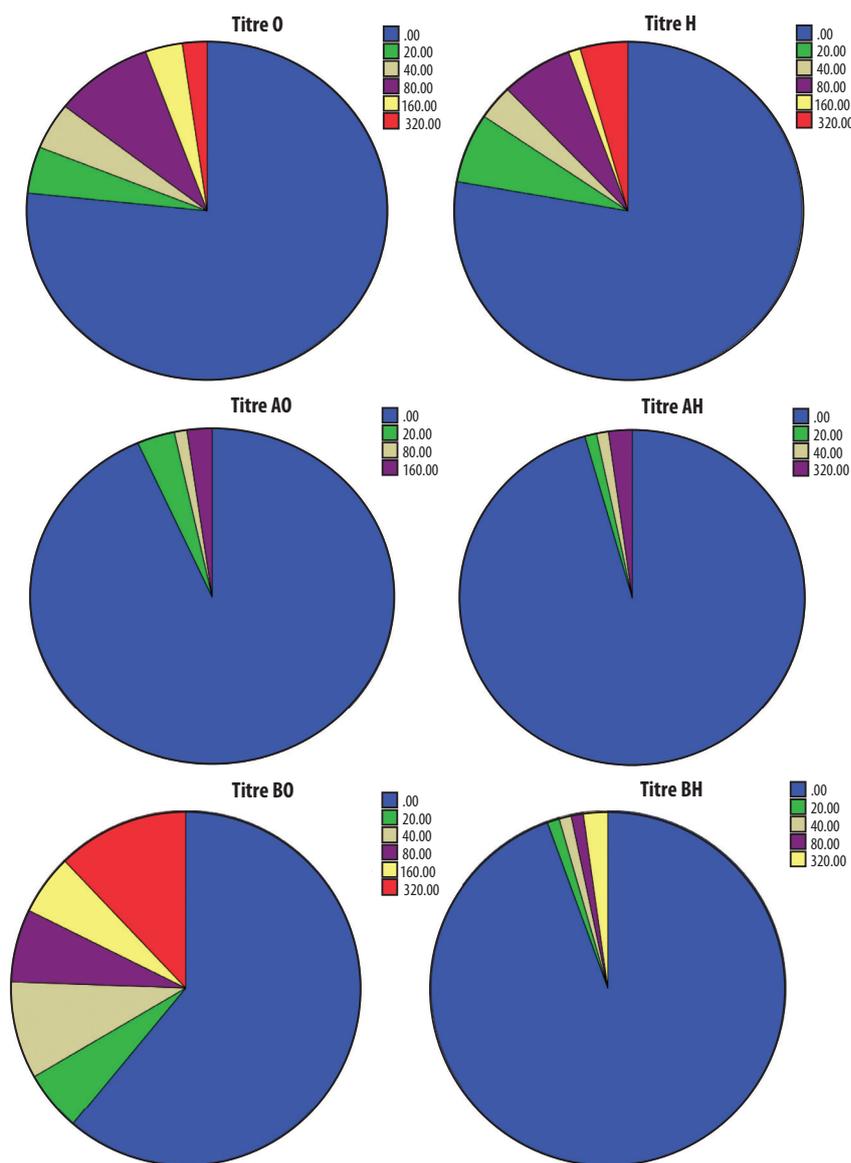


Fig. 1 Distribution of positive cases according to the titre of agglutinins.

Table 3. Cross tabulation of agglutinins titre with sex

Agglutinins	Female			Male		
	<1:20 N (%)	≥1:20 N (%)	Total	<1:20 N (%)	≥1:20 N (%)	Total
O	50 (83.3)	10 (16.6)	60	19 (63.3)	11 (36.6%)	30
Fisher's exact test	0.03*					
H	50 (83.3)	10 (16.6)	60	20 (66.66)	10 (33.3)	30
Fisher's exact test	0.06					
AO	58 (96.6)	2 (3.3)	60	26 (86.6)	4 (13.3)	30
Fisher's exact test	0.09					
AH	58 (96.6)	2 (3.3)	60	29 (96.6)	1 (3.3)	30
Fisher's exact test	0.7					
BO	47 (78.3)	13 (21.6)	60	13 (43.3)	17 (56.6)	30
Fisher's exact test	0.001*					
BH	57 (95)	3 (5)	60	28 (93.3)	2 (6.6)	30
Fisher's exact test	0.5					

*Statistically significant difference.

Table 4. Correlations among agglutinins

		Titre O						Total
		0.00	20.00	40.00	80.00	160.00	320.00	
Titre H	0.00	64	2	2	2	0	0	70
	20.00	1	2	1	2	0	0	6
	40.00	2	0	1	0	0	0	3
	80.00	0	0	0	4	2	0	6
	160.00	0	0	0	0	1	0	1
	320.00	2	0	0	0	0	2	4
Total		69	4	4	8	3	2	90
Correlation		r = 0.673*, P = 0.000						

		Titre O						Total
		0.00	0.00	0.00	0.00	0.00	0.00	
Titre AO	0.00	68	2	4	6	2	2	84
	20.00	0	2	0	1	0	0	3
	80.00	0	0	0	0	1	0	1
	160.00	1	0	0	1	0	0	2
Total		69	4	4	8	3	2	90
Correlation		r = 0.356*, P = 0.001						

		Titre O						Total
		0.00	20.00	40.00	80.00	160.00	320.00	
Titre AH	0.00	69	4	3	7	2	1	86
	20.00	0	0	0	0	0	1	1
	40.00	0	0	0	1	0	0	1
	320.00	0	0	1	0	1	0	2
Total		69	4	4	8	3	2	90
Correlation		r = 0.424*, P = 0.000						

		Titre O						Total
		0.00	20.00	40.00	80.00	160.00	320.00	
Titre BO	0.00	47	2	1	4	1	0	55
	20.00	3	1	1	0	0	0	5
	40.00	4	0	2	2	0	0	8
	80.00	2	1	0	1	2	0	6
	160.00	4	0	0	1	0	0	5
	320.00	9	0	0	0	0	2	11
Total		69	4	4	8	3	2	90
Correlation		r = 0.222*, P = 0.036						

		Titre O						Total
		0.00	20.00	40.00	80.00	160.00	320.00	
Titre BH	0.00	66	3	4	7	3	2	85
	20.00	1	0	0	0	0	0	1
	40.00	0	1	0	0	0	0	1
	80.00	1	0	0	0	0	0	1
	320.00	1	0	0	1	0	0	2
Total		69	4	4	8	3	2	90
Correlation		r = 0.079, P = 0.4						

*Statistically significant difference.

methods and criteria of interpretation etc.¹¹ In areas where fever due to infectious causes is a common occurrence, the possibility exists that false positive reactions may occur as a result of nontyphoidal fever. The purpose of the present study was to reappraise the diagnostic value of the Widal test by evaluating the basal antibody levels in the adult healthy population. In the current study, 69 (76.7%), 70 (77.7%), 84 (93.3%), 87 (96.7%), 60 (66.6%) and 85 (94.4%) of cases had antibody titres less than 1:20 for O, H, AO, AH, BO and BH, respectively. Whereas, 21 (23.3%) had anti-O agglutinins more than or equal to 1:20. In addition, antibody titre \geq 1:20 were seen in 21 (23.3%), 20 (22.2%), 6 (6.7%), 3 (3.3%), 30 (33.3%) and 5 (5.6%) for O, H, AO, AH, BO and BH, respectively.

There were significant differences between the presence of O, BO agglutinins with sex; the females had antibody titre more than male. This may reflect that females more prone to infection or because of the immune system of the females which results in more antibody titres.

Among 21 cases which had titre \geq 1:20 anti-O agglutinins, 15 had titre \geq 1:20 anti-H, 5 had titre \geq 1:20 anti-AO, 13 had titre \geq 1:20 anti-BO, 2 had titre \geq 1:20 anti-BH and 4 had titre \geq 1:20 anti-AH agglutinins. There were positive correlation between the presence of O agglutinins and H, AO, AH, BO agglutinins. This means that the increase levels of O agglutinins is accompanied by the increase of other types of agglutinins.

The cutoff values for O and H is 1:160, 1:40 for AO, 1:80 for AH and BH and 1:320 for BO, and thus considered to be baseline titre for these agglutinins in this region. Any titre above these would be significant and indicative of enteric fever.¹² Our results is in agreement with study carried out in Baghdad,¹³ the high level of agglutinins titres in our region among population might possibly be due to that the population is permanently “immunologically sensitized” due to constant exposure, the response to infection is more rapid, reaching higher levels and is less likely to be affected by antibiotic use when compared to non-endemic.¹⁴ Several studies support that re-evaluation of the Widal of the Widal baseline titre for healthy individuals should be done at regular intervals.¹⁴

Conclusion

Titre of 1:160 for anti-O and H agglutinins is considered as baseline titre for these

Table 5. Cutoff value

	Titre O	Titre H	Titre AO	Titre AH	Titre BO	Titre BH
Mean	22.2222	24.0000	5.1111	7.7778	58.0000	8.6667
SD	57.70475	69.42460	25.18446	47.56492	106.25600	48.16171

agglutinins in this region. Knowledge of endemic titre and proper interpretation of Widal test are necessary for accurate diagnosis of enteric fever to avoid misuse of antibiotics, thereby preventing the occurrence of drug resistance. ■

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