Original paper

Prevalence of Positive Widal Test among Healthy Personnel in Kerbala

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Abstract

Background: Typhoid and paratyphoid fever is still an existing health problem in many developing countries**.** Widal test is almost the most widely used method for diagnosis of typhoid fever in many developing countries including Iraq, though it is nonspecific with many drawbacks on its use.
Objectives: To estimate the prevalence of Widal test positivity in sera of healthy young personnel in Kerbala city.
Methods: A cross sectional study conducted in Karbala city, Blood samples from 107 healthy young adults aged 18-40 years old collected, whom are free of sign and symptoms, and not having significant ill health within last two months or typhoid fever within last six months.
Results and Discussion: males composed 48.6% of the sample, their mean age was 22.8 ± 3.66 years, and 51.4% of them had a titer of 1/80 for S.typhi O antibody, while other ABs titers were 44.9%, 47%, and 44.9% for S typhi H, S. paratyphi BO and S. paratyphi BH respectively. On other hand ABs titers ≥ 1/160 (positive) were found in 42.1 % for S. typhi O, while others were 28%, 23.4%, 20.6% for S. paratyphi BO, S. typhi H and S. paratyphi BH respectively. There was no statistical significant difference between those tested positive and the negative group in regards to gender or age, or WBCs count.
Conclusions: ABs titers are so high, that increases the doubts, and uselessness of the use of Widal test for diagnosis of typhoid fever, and raises the need for the estimation of basic titers for these ABs among Iraqi population.
Key Words: Widal test, Typhoid fever, Paratyphoid, Salmonella enterica, Kerbala, Iraq

Introduction

Enteric fever including typhoid and paratyphoid fever is still an existing health problem in many developing countries, with World Health Organization estimate of nearly 17 million infections and more than 150.000 deaths occurs each year worldwide, and the prevalence is directly related to unsafe water use, improper sanitation and poor hygiene practices at individual and community levels [(1-6)].
Typhoid fever which is a systemic disease characterized by febrile illness lasting for several days caused by some Salmonella(S) enterica subspecies mainly including Salmonella typhi, S. paratyphi A, S. paratyphi B, other signs and symptoms are nonspecific including headache, abdominal pain and leukopenia [(7-10)].
While diagnosis of typhoid fever depending merely on clinical presentation is non-convenient any more, the diagnosis of it is depending on different laboratory approaches including bacterial culture, serological markers and antigen detection is still difficult, as being non-reliable or non-applicable in many developing countries including Iraq [(9, 11-14)].

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The absolute diagnosis of typhoid fevers is by isolation of bacteria from blood, stool, urine or bone marrow cultures [3, 5, 11, 15, 16]. However the isolation of the bacteria by culture is very limited for various reasons including non-availability, time consuming, improper techniques use, non-trust of physicians with lab results and also due to the wide spread misuse and self-administration of antibiotics by patients and people in Iraq and many other countries as indicated by many researchers [3-5, 15].

Widal test is almost the most widely used method for diagnosis of typhoid fever in many developing countries [17-21], though it's an old agglutination test that been used for more than 100 years [5, 9, 16-18, 22]. Even with the drawbacks of Widal test including its non-specificity, cross-reactivity with other infectious agents, high false positives and high false negatives and cross but it continue to be used quite a lot, as no simple, reliable and applicable alternative methods available and because it's a cheap, traditional, easy, rapid and available method [2, 3, 16, 20, 23-26].

Also Widal test and antibodies titers for both O and H varies greatly in relation to level of endemicity of the typhoid fever as well as some other endemic diseases including tuberculosis, and there are many studies tries to set a basic titer for widal agglutination in different communities [2, 3, 5, 12].

In Iraq, till recent years, Widal test is the most widely used test for diagnosis of typhoid and paratyphoid fever in association with clinical presentations with or without white blood cell count to indicate leukopenia which is also another nonspecific sign for typhoid infection. In Iraq, and more specifically in Kerbala, no baseline titer for typhoid antibodies had been documented [27].

However many physicians in Iraq as a trend, with a clinical presentation suggesting typhoid fever, were considering an antibody (AB) titer of 1/160 or higher as a positive for typhoid fever (28-31), specifically for the AB O which is more associated with acute infection[11], accompanying leukopenia will be more convincing.

However, many people who complain of a moderate headache or 1-2 days fever could be sent to or do the test by their own in private laboratories, which frequently appear positive and patients make a provisional diagnosis of typhoid for the physician. The physicians will have difficulty to convince them that they do not have typhoid especially with non-availability of a definite diagnostic test, as blood or stool culture is time consuming, quite difficult to achieve for different reasons. Also many of those patients diagnose themselves already and describe their condition as a recurrence of typhoid, that attack them almost every year while they could have a viral infection or a heat stroke, the latter is a common false belief in Iraq that exposure to the hot sun causes typhoid fever.

Until recent years, an antigen- antibody (IgE and IgM) test for typhoid was available in Iraq, and it had been used increasingly in diagnosis of typhoid fever, and its accuracy is a matter of concern and need to be evaluated.

This study aims to estimate the prevalence of Widal test positives in blood of healthy young personnel in Kerbala city.

Methods

A cross sectional study conducted in Kerbala city, blood samples collection done for the period from April10-30, 2010. Blood samples from 107 healthy young adults aged 18-40 years old, whom agreed to participate in study, and signed consents to donate extra 2 ml of their blood for the study issue after the objectives of study been explained for them. They were medical personnel, students, or staff whom work or study in Kerbala College of Medicine, and were already giving blood samples for a screening tests for viral hepatitis and HIV prior to hepatitis B

vaccination. None of them was complaining of fever, specific headache or any significant ill health within last two months or typhoid fever within last six months.

Standard Widal tube agglutination test method used, serum dilution with saline were made for each antigen, and one drop each set add one drop of the Widal test antigen for S.typhi O (TO) S.typhi H (TH) S. paratyphi O (BO) and S. paratyphi H (BH) suspension added, mixed and incubated at 50 o C for 2 hours. After that agglutination observed and a negative test result considered when levels are less than1/80 of the corresponding antibody in the patient serum. A titer of 1/80, 1/160 and 1/320 or more considered as clinically significant and registered as positive, a titer of 1/160 or more was considered positive. White blood cells count (WBC) was measured using manual count in chamber technique.

Data were entered and analyzed using SPSS (Statistical package for social science) program version 15, Chi square test used for analysis of qualitative data, and Student's t test used for analysis of quantitative data. Statistical significance was considered when p value was less than 0.05.

Results

Of total 107 persons, males were 52 (48.6%) and females were 55 (51.4%) of the sample. Their age range from 19-39 years with a mean ± standard deviation (SD) of 22.8 ± 3.66 years, 51.4% of the them had a titer of ≥ 1/80 for TO antibody, other ABs titers were 44.9%, 47%, 44.9% for TH, BO and BH respectively and table 1 shows the details of ABs titer. While ABs titers ≥ 1/160 (positive) were found in 42.1 % for TO, while others were 28%, 23.4%, 20.6% for BO, TH and BH respectively as shown in figure 1. There was no statistical significant difference between those tested positive and the negative group in regards to gender, age, or WBCs count, though positive Widal results were slightly higher among females, and those tests positive had slightly higher WBC count as shown in tables 2, 3 and 4.

Table 1. Antibodies titers for different Salmonella species

	Antibody Titer								
Antibody	Negative		1/80		1/160		1/320		Total
	N	%	N	%	N	%	N	%	
S. typhi O (TO)	52	48.6%	10	9.3%	25	23.4%	20	18.7%	107
S. typhi H (TH)	60	56.1%	22	20.6%	6	5.6%	19	17.8%	107
S. paratyphi BO (BO)	61	57%	16	15%	13	12.1%	17	15.9%	107
S. paratyphi BH (BH)	60	56.1%	25	23.4%	12	11.2%	10	9.3%	107

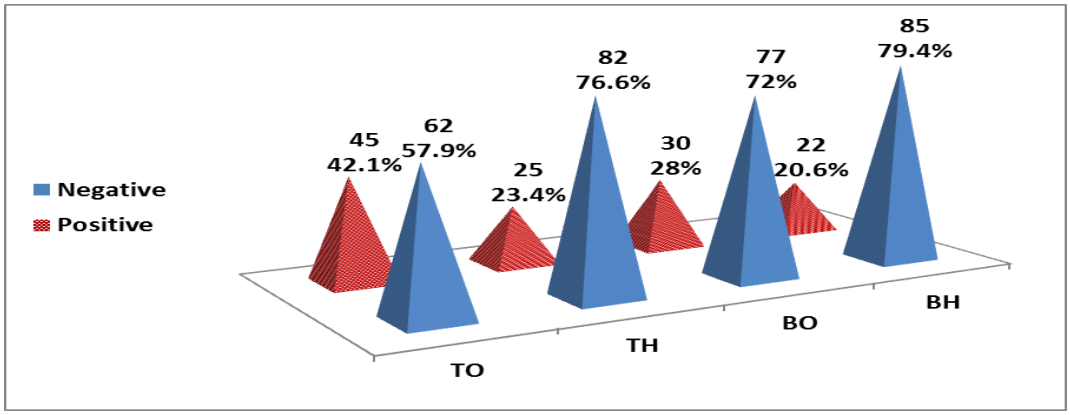

Figure 1. prevalence of Widal test positive (≥ 1/160) for various serotypes

Table 2. Relation between gender and Widal test results for various serotypes

		Gender						P value
		Male		Female		Total		
		N	%	N	%	N	%	
		52	48.6	55	51.4%	107	100%	
TO	Positive	20	38.5%	25	45.5%	45	42.1%	0.557
	Negative	32	61.5%	30	54.5%	62	57.9%	
TH	Positive	11	21.2%	14	25.5%	25	23.4%	0.652
	Negative	41	78.8%	41	74.5%	82	76.6%	
BO	Positive	13	25.0%	17	30.9%	30	28.0%	0.526
	Negative	39	75.0%	38	69.1%	77	72.0%	
BH	Positive	10	19.2%	12	21.8%	22	20.6%	0.813
	Negative	42	80.8%	43	78.2%	85	79.4%	

Table 3. Relation between age and Widal test results for various serotypes

		Age in years			P value
		N	Mean	SD	
		107	22.80	3.67	
TO	Positive	45	22.49	4.10	0.452
	Negative	62	23.03	3.33	
TH	Positive	25	23.32	4.64	0.424
	Negative	82	22.65	3.33	
BO	Positive	30	21.73	1.46	0.059
	Negative	77	23.22	4.16	
BH	Positive	22	22.05	1.39	0.278
	Negative	85	23.00	4.04	

Table 4. Relation between WBC count and Widal test results for various serotypes

		WBCs /mm^3			P value
		N	Mean	SD	
		107	7038.3	1966.0	
TO	Positive	45	6920.0	1884.8	0.598
	Negative	62	7124.2	2033.7	
TH	Positive	25	6852.0	1952.2	0.591
	Negative	82	7095.1	1978.6	
BO	Positive	30	6790.0	2071.9	0.417
	Negative	77	7135.1	1928.4	
BH	Positive	22	6868.2	2280.6	0.651
	Negative	85	7082.4	1888.8	

Discussion

Diagnosis of typhoid fever infection is a real problem for physicians in Iraq, and giving a negative diagnosis for it is another problem, as the difficulty of getting an accurate or reliable diagnostic test for such as blood or stool cultures. This condition is more complicated by the misuse or habitual use of antibiotics by patients and also by the habitual or misuse of Widal test for diagnosis of typhoid fever, so we were trying to evaluate the prevalence of positive Widal test among young, healthy and symptoms free individuals.

As appeared in our result the AB TO titer was the most prevalent AB, were 51.2% have elevated titer (≥1/80), other AB titers were slightly less but still clearly elevated.

As mentioned above, ABs titers were considered positive if it was ≥ 1/160, that appeared in 42.1 % for TO, while others were 28%, 23.4%, 20.6% for BO, TH and BH respectively. These percentages are much higher than recorded among healthy blood donors in Baquba city\Iraq by Hasan

et al 2011 for ABs of TO, BO, and BH which were 11.3%, 11.3%, 10.6% respectively[27]. While same study recorded a higher titer for TH of 36.3% than our result, and more than triple other ABs titers in their study [27].

These titers are higher than other studies done elsewhere on healthy populations, as Willke et al in Turkey who indicated a titers of 7%, 2% for TO and TH respectively in healthy population[8]. also several studies in India showed lower AB titers, including Bijapur et al 2014 in North Kerala India whom documented that 3.2% , 3.6% had a titer of 1/80 or more for TO, TH respectively, and 0% BO and BH [32]. In Uttarakhand, 2.3%, 29.2% had a titer of 1/80 or more for TO and TH respectively and 0% for BH [5], at Ahmednagar, Maharashtra, 41.7%, 27.2%, 7.8% had a titer of 1/80 or more for TO, TH and BH ABs respectively, while 21.4%, 15.5%, 1% had a titer of 1/160 or more for TO, TH and BH ABs respectively [15], and another study in India recorded a titer of 1/80 in 13.2% and only for TO AB [33].

In a study in Mexico city 5.5% had a titer of 1/80 or more for TO, were other ABs titers in less than 1% of the sample[34], while a study in Nigeria 13.8% of the healthy subjects had TO and 18.5% had TH in their serum at a titer of 1/80 or more [35].

Its shown that our results is higher than other studies in different regions of world, and already there were wide differences in titers even within same countries, this could be related to endemicity difference, or could be due to different techniques and material used, that is another problem with the Widal test [5, 14, 16, 17].

In our study there was no significant association in regards to age, gender and WBC count. However, slightly lower WBC count was found among those with higher titer, and the test positive were slightly higher among females. These results showed clearly that the use of Widal test for diagnosis or screening of typhoid fever is non-reliable anymore even with the raise of the cut-off titer to 1/320 [36], and it's a misleading test.

In conclusion AB titers for different Salmonella species is quite prevalent among healthy population in Karbala, and high percent of false positive Widal test, this makes the relay on Widal test for diagnosis of typhoid is very weak, and nonscientific even at higher levels of 1/320 or higher and there is a need for another effective specific and applicable tests to be introduced and evaluated, such as cultures, PCR and others, as well as the need to estimate basic Salmonella ABs titers among Iraqi population.

Acknowledgments: we would like to thank paramedical staff in microbiology and hematology units in the central laboratory of Al-Hussein Teaching Hospital in Kerbala for their help in conducting the tests.

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