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RESEARCH ARTICLE

Comparison of widal agglutination test with ELISA typhi test for serological diagnosis of typhoid fever in some Iraqi patients in Diyala governorate

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Abstract

Typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries , this study was aimed to compare between ELISA test and widal test for diagnosis typhoid fever in some Iraqi patients in diyala governorate. A total of (120) Iraqi patients infected with typhoid fever were involved in this study , these patients attended to inflammatory bowel disease clinic of Baquba Teaching Hospital during the period from mid- July 2012 to mid-June 2013. Their ages ranged between (10 - 63) year. These patients included 77 males and 43 females , also forty apparently healthy individuals with age range from (11 - 58) year were studied as control group, this group included 25 males and 15 females. A total of 44 , 57 , 12 , 7 out of the 120 patients had significant O agglutination titer value (1:160, 1:320 , 1:640 , 1:1280) respectively , on the other hand , a total of 41 , 52 , 18 , 9 out of the 120 patients had significant H agglutination titer value (1:160, 1:320 , 1:640 , 1:1280 respectively). In this study, two methods were used for diagnosis of typhoid fever . The first method was using widal test , 120 sera samples of typhoid fever patients were found to be positive for this test (i.e. 100 %) , on the other hand ELISA typhi test was using for detection of *Salmonella typhi* antibody (St-Ab) , 64 out of 120 sera samples of typhoid fever patients were found to be positive for this test (i.e. 53.3 %) , with highly significant difference was noticed between them ($P<0.001$). In conclusion, the specificity and sensitivity of ELISA typhi test was higher than widal test and more accurate method for detection *Salmonella typhi* antibody, also well suitable for routine determination of typhoid fever .

Introduction

Typhoid fever is a severe multi-systemic illness characterized by the classic prolonged fever, sustained bacteremia without endothelial or endocardial involvement and bacterial invasion of and multiplication within the mononuclear phagocytic cells of the liver, spleen, lymph nodes and Peyer.s patches . Typhoid fever is potentially fatal if untreated. It is caused by *Salmonella typhi* a Gram negative bacilli (1,2). Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with other febrile infections. Incidence of typhoid fever has been estimated at approximately 22 million cases with at least 200,000 deaths occurring worldwide annually , the disease is endemic in south-east , far-east and middle east Asia, as well as in Africa and central and south America (3,4). The disease is unique to human, it is characteraised by malaise, fever, abdominal discomfort, transient rash, splenomegaly, hepatomegaly, bradycardia, and leucopenia, the most prominent major complications are intestinal hemorrhage, and perforation. Infection occurs in all age groups with a higher incidence and more variable clinical presentation in children (5). Humans are infected through eating raw or undercooked foods, including meat, poultry, egg and dairy products (6) .*Salmonella* serotypes including *Salmonella typhi, S. paratyphi A, S. paratyphi B and*

S. sendai. During the course of infection, the causative bacilli can be isolated from feces, urine, bone marrow and blood (7). Accurate diagnosis of typhoid fever at an early stage is important not only for etiological diagnosis, but also to identify individuals that may serve as a potential carriers, who may be responsible for acute typhoid fever outbreaks (8). Different techniques are used for the diagnosis of typhoid, including blood culture, bone marrow culture, rectal swab culture, urine culture, widal test, ELISA, and immunofluorescence (9). The widal test is a presumptive serological test for typhoid fever , in case of *Salmonella* infections, it is a demonstration of the presence of O-soma false-positive result. widal test is of little clinical relevance due to the number of cross reacting infections and relatively low sensitivity and specificity(10). There have been efforts to develop faster and more sensitive and specific serological assays for the diagnosis of typhoid fever, so recently ,various immunoassays such as ELISA and immunofluorescence which are used to detect *S. typhi* antigens or antibodies (11) , providing greater sensitivity and dramatically speeding up detection. Furthermore provides critical information to public health decision makers with respect to clinical management, disease prevention, and infection control strategies. One of the major drawback of widal test is cross-reactivity due to which some other bacteria of same genus often produces false positive results , ELISA Typhi test is another test used to the diagnosis of typhoid fever (12). As for Iraq especially in diayla governorate , there is no previous study on the use of ELISA technique in the diagnosis of typhoid fever , therefore the aim of this study is to compare between ELISA test and widal test for diagnosis typhoid fever in some Iraqi patients in diyala governorate.

Subjects and Methods

Subjects

Patients Study Group

A total of (120) Iraqi patients infected with typhoid fever were involved in this study , these patients attended to inflammatory bowel disease clinic of Baquba Teaching Hospital during the period from mid- July 2012 to mid-June 2013. Their ages ranged between (10 - 63) year. These patients included 77 males and 43 females. They were sequentially selected from cases referred to the hospital at first presentation. They were diagnosis based upon the standard clinical features , physical examination and laboratory test (widal test) .

Control group

Forty apparently healthy individuals with age range from (11 - 58) year were studied as control group. This group included 25 males and 15 females. Samples were collected from those individuals with no history of infections in the recent past.

Specimens collection

From each individual included in this study, 5 ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes, it was left to stand at room temperature (20-25°C) to allow it to clot, then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

Methods

Widal Test

Principle of the assay

A rapid slide agglutination test using standard S. typhi O and H antigen suspensions .The widal test measures serum agglutinins against somatic and flagellar antigens. When an antibody combines with a corpuscular antigen (forming part of a cell - e.g. bacteria, or inert part with bound antigen) the cells agglutinate, that means they form clumps.(i.e. the clumping of cells such as bacteria in the presence of an antibody. The antibody or other molecule binds multiple particles and joins them, creating a large complex) (13) .

Assay procedure

1. By using microtiter pipette or 0.2 ml pipette, deliver 0 .32, 0.16 ,0.08, 0.04, 0.02, 0.01, 0.005 ml of undiluted serum into a row of 3-4 cm diameter circles on a white title.

2. one-drop (0.04 ml) of the appropriate well-shaken suspension was added to each serum aliquot.

3. Mix by stirring for a few seconds with a wooden applicator stick proceeding from the highest dilution (0.005-0.16 ml) serum, spreading the contents to fill the circles.

4. Rotate the tile slowly and read agglutination test with serum dilutions of 1:20, 1:40, 1:80, 1:160, 1:320 , 1:640 and 1:1280 respectively (13).

ELISA test for typhoid fever (Human salmonella typhi antibody (St-Ab) ELISA Kit.

This assay recognizes human salmonella typhi antibody (St-Ab).

Principle of the assay

The microtiter plate provided in this kit has been pre-coated with specific antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then added Horseradish Peroxidase (HRP)-conjugated -anti-human immunoglobulin to each well and incubate. Finally, substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Calculate the valence of human salmonella typhi antibody (St-Ab) in the samples (14).

Assay procedure

1. Set one Negative Control wells , one Positive Control wells. Set one Blank well (Add 100µl of Sample Diluent).
2. One hundred microliter of diluted Sample, positive control and negative control was added per well. Cover with the adhesive strip. Incubate for 30 minutes at 37°C.
3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with wash buffer (350µl) using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, stay for 30 seconds. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. One hundred microliter of HRP-conjugate to was added each well, not to Blank well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at 37° C.
5. The aspiration and wash was repeated three times as before.
6. Fifty microliter of substrate A and 50µl of substrate B was added to each well. Incubate for 10 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

7. Fifty microliter of stop solution was added to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. The optical density of each well was determined within 10 minutes, using a microplate reader set to 450 nm (14) .

Calculation of results

For calculation the valence of human salmonella typhi antibody (St-Ab), compare the sample well with control. (If the OD negative ＜0.1, calculate as 0.1).

While OD sample/ OD negative ≥ 2.1: Positive

While OD sample / OD negative ＜2.1: Negative

Results and Discussion

Today most of the burden of typhoid fever occurs in the developing world, where sanitary conditions remain poor ,typhoid fever is a major public health problem associated with significant morbidity and mortality in many countries (19). It is clear from table (1) that the majority of patients group and control group are the males [77 out of 120 and 25 out of 40 respectively] rather than the females [43 out of 120 and 15 out of 40 respectively] with highly significant differences between both frequencies (P<0.001). The ratio between male to female was 1.79:1 as regard to patients group and 1.67 : 1 as regard to control group. Both sexes can be infected by typhoid fever, this high frequency of infection with typhoid fever among males patients group may be attributed to socio-community nature of Iraqi people which makes men undergone the responsibility of working and eventually are in great contact with the pathogens rather than the women as well as the sex differences among patients group could be explained on the basis that males may have a greater chance to come in contact with risk factors of typhoid fever than females. According to (15) , 61 % of the patients with typhoid fever are males, mainly 10 -30 years old, moreover , (16) reported that typhoid fever affect men 2 to 4 times more than women.

Table 1 : Distribution of the studied groups according to gender.

Sex	Patients		Control		C.S	P. value
	No.	%	No.	%	H.S	0.001
Female	43	35.9	15	35		
Male	77	64.1	25	65		
Total	120	100	40	100		
Male / Female ratio	1.79:1		1.67:1			

The distribution of studied groups according to age are shown in (table 2), the results of this study recorded that the age ranged between (10 – 63 year) (mean of 32.21 year) among patients group and more than half of patients (51.7 %) are located within first and second decade (10- 29 year) whereas the age ranged between (11 – 58 year) (mean of 28.15 year) among control group, moreover the more than half of control group (62.5 %) are also located within first and second decade (10- 29 year). These results

coincide with the previous study done in kuala-lumpur as (15) who establish that (32. 4 year) was the mean age for patients having typhoid fever , also other study was done by (17) registered that the mean age for persons having of typhoid fever was (33.2 year) .Moreover the patients group age range between (10 – 19 year) recorded high percentage of positivity (22.9 %). Infection occurs in all age groups with a higher incidence and more variable clinical presentation in children (15).

Table 2 : Distribution of the studied groups according to age

Age groups	Patients		Control	
	No.	%	No.	%
(10 -19)	35	29.2	9	22.5
(20 -29)	27	22.5	16	40.0
(30 - 39)	20	16.6	6	15.0
(40 - 49)	16	13.3	5	12.5
(50 - 59)	14	11.7	4	10.0
60 +	8	6.7	---	---
Total	120	100.0	40	100.0
Mean	32.21 year		28.15 year	

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses .Sero-diagnosis of typhoid fever has been attempted since the late nineteenth century by Widal and Secard (1). Sero-diagnosis of typhoid fever by widal test based on demonstrating the presence of agglutinins (antibodies) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* (20).

The results of the widal agglutination test in this study are presented in (table 3). Titer values from 1:160 and above were regarded as significant and therefore positive for the Salmonella antigen. A total of 44 , 57 , 12 , 7 out of the 120 patients had significant O agglutination titer value (1:160, 1:320 , 1:640 , 1:1280 respectively) and therefore were regarded as positive , on the other hand , a total of 41 , 52 , 18 , 9 out of the 120 patients had significant H agglutination titer value (1:160, 1:320 , 1:640 , 1:1280 respectively) and therefore were regarded as positive (table 3).

There is no consensus in literatures concerning the diagnostic criteria for interpreting widal test (20). In Iraq many studies were done and various titers are stated, (21) considered an initial titer of 1/160 is the most reliable one in the consideration of typhoid fever, (22) considered an initial titer of 1/320 as diagnostic value, (23) mentioned that a titer 1/160 is highly specific but less sensitive in the community of Basrah, (24) considered the titer of 1/160 and 1/320 for O and H respectively are diagnostic value. The earliest serological response in acute typhoid fever is said to be arise in the titer of the O antibody, the H antibody usually develops more slowly but persists longer than O, towards the end of the first week of illness titers of O antibodies may be as high as 1:160 (26) . In this study, two methods were used for diagnosis of typhoid fever . The first method was using widal test for detection of somatic (O) and flagellar (H) agglutinins . 120 out of 120 sera samples of typhoid fever patients (i.e. 100 %) were found to be positive for this test, on the other hand ELISA typhi test for detection of human *Salmonella typhi* antibody (St-Ab) has been applied . 64 out of 120 sera samples of typhoid fever patients (i.e. 53.3 %) were found to be positive for this test , with highly significant difference was noticed between them ($P<0.001$) as shown in (table 4) .These findings reflected highly sensitivity and specificity of ELISA typhi test for detection of human *Salmonella typhi* antibody (St-Ab) (i.e. more accurate method for detection of St-Ab) , therefore the current results confirm that a ELISA typhi test is more sensitive and specific manner to detect St-Ab than widal test.

Table (3) : Anti- *S typhi* O and H antibody titers in sera of patients detected by Widal test

Widal test	Patient group No.120							
Ab titer to salmonella typhi O	1:160		1:320		1:640		1:1280	
	no	%	no	%	no	%	no	%
	44	36.7	57	47.5	12	10.0	7	5.8

Ab titer to salmonella typhi H	**1:160**		**1:320**		**1:640**		**1:1280**	
	no	**%**	**no**	**%**	**no**	**%**	**no**	**%**
	41	34.2	52	43.3	18	15	9	7.5

*control group for this study, analysis of Salmonella typhi antibody titers for both types (O and H) had been performed and shows that the Widal test in the normal individual was negative in all 40 cases .

Table 4 : Comparison between widal test and ELISA typhi test for diagnosis typhoid fever

Patient No (120)	**Test**	**Positive (+ve)**	**Negative (-ve)**	**P. value**	**C.S**
	Widal test	120 (100.0 %)	0.0 (0.0 %)	0.001	H.S
	ELISA typhi test	64 (53.3 %)	56 (46.7 %)		

*control group for this study, analysis of human salmonella typhi antibody (St-Ab) had been performed and shows that the ELISA typhi test in the normal individual was negative in all 40 cases .

Our result is in agreement with the result obtained by (20). Additionally, the results of this study agreed with (16) who reported that a significant difference has been noticed between widal test and ELISA for detection of typhoid fever . Moreover (27) has found that in young adults, a considerable difference among widal and ELISA for investigating typhoid fever**,** also found that ELISA was not only relatively rapid but also more accurate than traditional method (widal test) for the detection of *Salmonella typhi* antibody .

Widal test has been used for over a century in developing countries for diagnosing typhoid fever but it has a low sensitivity, specificity and positive predictive value, which changes with the geographical areas. Sharing of O and H antigens by other *Salmonella* serotypes and other members of *Enterobacteriaceae* makes the role of widal test even more controversial in diagnosing typhoid fever, also the result of widal test is also effected by variables such as frequency distribution of agglutinins in the population, effect of antibiotic treatment and antibody response to enteric fever (1,27). Additionally , false positive results are seen with patients who had previous vaccination or infection or reported in association with a few autoimmune diseases, beside that false negative results may be associated with early treatment, with hidden organisms in bone and joints and with relapse of typhoid fever (28). In conclusion, the specificity and sensitivity of ELISA typhi test was higher than widal test , as a result, the ELISA typhi test was accurate, precise, objective, inexpensive and well suitable for routine determination of typhoid fever .

References

1-Parry ,C.M. ; Hien, M.B. ; Dougan, M.D. ;White, N.J. ; and Farrar, M.T.(2002). Typhoid fever. *N Engl J Med*, 347:1770–1782.

2- Andews-polymens, H.and Baumter, A.J. (2006). Pathogenomics of salmonella species, in pathogenomics. Hacker, J. Dobrinoff, U.(eds) Weinheim, Wiley-VCH, . pp.109-124.

3- World Health Organization. (2006). 6th International Conference on Typhoid Fever and other Salmonellosis. Geneva, WHO.

4- Crump, J.A. ; Stephen , P. and Luby, E.D. (2004). The global burden of typhoid fever. Bull World Health Organ.; 82:1-24.

5-Ghenghesh ,K.S. ; Franka, E. ; Tawil, K.; Wasfy ,M.O.; Ahmed, S.F.; Rubino, S. and Klena, J.D. (2009). Enteric Fever in Mediterranean North Africa. *J Infect Dvl Ctries*.; 3: 753-761.

6- Baumler, A.J. ; Hargis, B.M. and Tsolis, R.M. (2000) . Tracing the origins of Salmonella outbreaks. Science;287:50– 52.

7- Tirado, C. and Schmidt, K. (2001) .WHO surveillance program for control of food borne infections and intoxications. *Europe. J Infect* . ;43:80–84.

8- Kingsley, R.A. and Baumler, A.J. (2000). Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. *Mol. Microbiol.*; 36, 1006–1014.

9- Crump,J.A.; Eric, D. ; Mintz,E.D. ;Whitaker,J.A. ;Gilman,R.H and Olsen,S.J. (2010). Global Trends Typhoid and paratyphoid fever. *Clini. Infect. Dis.;* 50:241-6.

10- House, D.; Wain, J. ; Ho ,V.A. ; Diep, T.S. ; Chinh, N.T. ; Bay ,B.V. ; Parry, C.M. Dougan, G. (2001). Serology of Typhoid Fever in an Area of Endemicity and Its Relevance to Diagnosis. *J Clin Microbio*.; 39: 1002-1007.

11- Chart , H. ; Cheasty, T. ; de Pinna, E. ; Siorvanes, L. ; Wain, J. ; Alam, D. ; Nizami , Q. and Bhutta, Z. (2007) . Serodiagnosis of *Salmonella enterica* serovar Typhi and *S. enterica* serovars Paratyphi A, B and C human infections. *J Med Microbiol*.; 56: 1161-1166.

12- Bhutta, Z.A and Dewraj , H.L. (2006) . Current concepts in the diagnosis and treatment of typhoid fever. *BMJ* .; 333:78-82.

13- Olopoenia, L.A. and King, A .L. (2004) . Widal agglutination test. 100 years later, St, Plagued by controversy, Post graduate Medical J.; 760: 80-84, Pub Med (10644383).

14- Olsen, S.J. ; Pruckler, J. ; Bibb, W.; Nguyen, T.M. ; Tran, M.T.; Nguyen, T.M. ; Sivapalasingam, S. and Gupta, A. (2004). Evaluation of rapid diagnostic tests for typhoid fever. *J Clin Microbiol.*; 42:1885-1889.

15- Abdullahi, M. (2010). Incidence and antimicrobial susceptibility pattern of salmonella species in children attending some hospitals in kano metropolis ,kano state – Nigeria. *Bayero J.of Pure and App. Sci.* ; 3(1): 202 – 206.

16- Gopalakrishnan, V .; Sekhar, W .Y. ; Soo, E. H.; Vinsent, R. A. and Devi ,S. (2002). Typhoid fever in kuala lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella typhi* . *Singapore Med. J .;* 43(7) : 354-358.

17- Jesudason, M.V. and Sivakumar, S .(2006). Prospective evaluation of a rapid diagnostic test Typhidot for typhoid fever. *Indian J Med Res*.; 123: 513-516.

18- Wafaa , M.K. ; Laila , A. ; El attar, M. ; Ashour , S . ; Ayman , M. (2011). The dilemma of widal test - which brand to use? a study of four different widal brands: across sectional comparative study. *Annals of Clinic Microb. and Antimi.;* 10:7.

19- Okafor, A.I. (2007). Haematological alterations due to typhoid fever in Enugu Urban- Nigeria. Malaysian J. of Micro.; 3(2) : 19-22

20- Aziz , T and Haque ,S.S . (2012). Role of widal test in the diagnosis of typhoid fever in context to other test . *American J. of Bio.;* 2(1): 16-18 .

21- Al- Rawi, D. T. (1992). Evaluation of the Widal test in the diagnosis of typhoid fever. *M.Sc. Thesis,* College of Medicine, Baghdad University.

22- Al-Abbasi, A. M. and Al-Obaidi, S.(1994) . the level of *Salmonella typhi* antibodies among the Iraqi Subjects and their value in the diagnosis of typhoid fever. *J Comm Med*.; 7 (2): 113-19.

23-Salah, M. (1986). Typhoid fever: immune response in patient, vaccines and the diagnostic value of single Widal test in Basrah community. *M.Sc. Thesis*, College of Medicine, University of Baghdad.

24- Ali, H. I. (1993). Enteric fever clinical and diagnostic evaluation. *M.Sc. Thesis*, College of Medicine, University of Baghdad.

25- Sherwal , B .L. ; Randhawa , V.S. ; Jais, M. ; Dhamija, R.K. ; Kaintura, A. and Kumar, M.(2004). A comparative study of typhidot and widal test in patients of typhoid fever. *JIACM.*; 5(3): 244-246.

26- Saha, M.R. ; Dutta , P. ;Palit, A. ; Dutta, D. ;Bhattacharya, M.K. ; Mittra, U. and Bhattacharya, S. K. (2003) . A Note on Incidence of Typhoid Fever in Diverse Age Groups in Kolkata, India. Jpn. *J. Infect. Dis.;* 56;121-122.

27- Moustafa , A. ; Fadeel, L.; Momtaz, M.; John, D. ; Engy, E. ; Mayar, M. ; Mohamed, A. ; Bassem, A. (2011). Evaluation of a newly developed ELISA against Widal, TUBEX-TF and Typhidot for typhoid fever surveillance . *J Infect Dev Ctries*; 5(3): 169-175.

28- Singh, S.(2001). Symposium : Typhoid fever-Pathogenesis and Laboratory Diagnosis. *J. Indian Academy of Clinic. Med. .;* 2: 17-20.