Detection of *Salmonella typhi* agglutinins in sera of patients with other febrile illnesses and healthy individuals

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**Abstract**

**Background and Purpose:** Widal test is frequently applied for the detection of *Salmonella* agglutinins to diagnose *Salmonella enterica* serotype *Typhi* infection. There are however a number of controversies challenging the diagnostic utility of this test. This study was performed to determine the prevalence of *Salmonella* agglutinins in patients with other febrile illnesses and healthy blood donors.

**Materials and Methods:** Sera from 50 healthy blood donors were compared for the presence of *Salmonella* agglutinins in various groups of patients with other febrile illnesses using Widal test in the division of Serology and Immunology at King Khalid University Hospital, Riyadh. The patient groups of other febrile illnesses included infections with Beta-hemolytic streptococcus (n = 50), Brucella (n = 46), *Helicobacter pylori* (n = 24), *Treponema pallidum* (n = 30), *Toxoplasma* (n = 44), and other parasites (n = 20).

**Results:** Majority of the patients and normal individuals were tested positive for Widal test at dilution of less than 1 : 40 both for the O (62.5%) and H (64.6%) antigen. A decreasing trend in Widal reactivity was observed with increasing dilutions of the serum samples. At 1 : 160 titer, which is generally considered as a cut off point for positive Widal test, 6.4 and 11% individuals had positive Widal test for O and H *Salmonella* antigens, respectively.

**Conclusion:** Detection of a significant number of positive Widal tests in conditions where it is expected to be nonreactive appears to be a serious problem in making a correct diagnosis of typhoid fever, thus challenging the diagnostic utility of the Widal test.

**Keywords:** False positive, other febrile illnesses, *Salmonella typhi*, Widal test

**Résumé**

**Contexte et objet:** Widal test est souvent appliqué pour la détection de *Salmonella* agglutinines pour diagnostiquer la *Salmonella enterica* sérotype *Typhi* infection. Il y a cependant un certain nombre de controverses contestant l’utilitaire de diagnostic de ce test. Cette étude a été effectuée afin de déterminer la prévalence de *Salmonella* agglutinines chez les patients atteints d’autres maladies fébriles et donneurs de sang.

**Méthodes et matériaux:** La présence de Salmonella agglutinines dans divers groupes de patients, de donneurs de sang sain sérums de 50 ont été comparés autres maladies fébriles à l’aide de test Widal dans la division de la sérologie et d’immunologie à l’hôpital universitaire du Roi Khaled, Riyadh. Les groupes de patients d’autres maladies fébriles inclus des infections à streptococcus bêta-hémolytique (n = 50), Brucella (n = 46), *Helicobacter pylori*(n = 24), *Treponema pallidum*(n = 30), *Toxoplasma*(n = 44) et autres parasites (n = 20).

**Résultats:** La majorité des patients et normale particuliers ont été testés positifs pour le test de Widal à dilution de
Introduction

Despite the introduction of several serological tests,\(^{[1-7]}\) Widal test is commonly applied for diagnosis of typhoid fever. As the typhi antigen is shared by a large number of organisms from the Salmonella genus and other related organisms,\(^{[1,2,8]}\) a positive Widal test is therefore likely to occur in several conditions other than the actual Salmonella enterica infection. False positive Widal test has already been reported in other febrile illnesses such as malaria, tuberculosis, and schistosomiasis.\(^{[4,5,12]}\) This study was performed to assess the reactivity of Widal test by detecting Salmonella agglutinins in other febrile conditions or in otherwise healthy individuals.

Materials and Methods

Study population

Sera from 264 individuals comprising of 214 patients with other febrile illnesses and 50 healthy blood donors were tested for the presence of Salmonella agglutinins using Widal test. The patient groups included 50 patients with confirmed diagnosis of infection with Beta-hemolytic streptococi, with positive titers for antistreptolysin O antibodies; 46 patients infected with Brucella abortus and melitensis spp.; 44 patients tested positive for Toxoplasma; 30 had a positive Venereal Disease Research Laboratory test, Treponema pallidum Haemagglutination assay, and Enzyme Link Immunosorbert Assay IgG (index>1.1); 24 were positive for Helicobacter pylori IgG and IgA; and 20 patients had evidence of various parasitic infections. Findings of each group of patients were compared with a group of 50 normal healthy individuals to match the number of individuals in the largest group of patients with hemolytic streptococcus infection. These individuals were also matched for age and gender to serve as normal controls. The inclusion criteria for the healthy blood donors were no history of typhoid infection or vaccination in the past and no other febrile illness for at least six months prior to testing.

Collection of blood sample

5 ml of blood was collected from each patient by venipuncture. Samples were left to clot and serum was collected, aliquoted, and kept at -20°C until used.

Widal tube agglutination test

The standard Widal tube agglutination test was performed using a commercial kit (Murex Biotech Limited, UK) in accordance with the manufacturer instructions. Saline serum dilutions were made for each antigen to be tested. One drop of bacterial suspension was added to each tube, contents were mixed and incubated at 37°C overnight for both O and H suspensions, and agglutination was observed at the end of the incubation. Based on the previous study in the Kingdom of Saudi Arabia,\(^{[13]}\) Widal test was interpreted as positive if the titer of agglutinins was either equal to or more than 1 : 160.

Results

Among the patients, there were 126 males and 88 females with the mean age of 27 ± 9 years. The normal healthy individuals comprised of 34 males and 16 females with the mean age of 25 ± 7 years. Table 1 describes the Widal reactivity in the patients with other febrile illnesses and the healthy normal donors. Generally, Widal reactivity for both O and H antigens was present in all the groups including normal healthy individuals. Majority of the individuals reacted at less than 1 : 40 dilution both for the O (62.5%) and H (64.4%) Salmonella antigens. A decreasing trend in Widal reactivity was observed with increasing dilutions. At 1 : 40 dilution, the O and H reactivity was 7.9 and 7.2%, respectively and at 1 : 160 titer, which is considered as clinically significant, Widal reactivity for O antigen was 6.4% and H antigen was 11% in all the groups including normal healthy individuals. Comparatively smaller percentage of individuals, 2.7 and 3.4% had a positive Widal test for O and H antigens respectively at a titer of 1 : 320. Among the groups at 1 : 160 dilution, the highest number of patients (eight patients) tested positive for H antigen were in the group infected with Toxoplasma, followed by patients infected with Brucella (four patients) and H. pylori (four patients). Among the healthy individuals, four tested positive for Salmonella H antigen. Similarly for O antigens, the highest number of positive Widal tests were detected.

Mots clés: Fausses positifs, d’autres fébriles maladies, Salmonella typhi, Widal test
Discussion

This study shows a significant number of patients suffering from other febrile illnesses, and normal individuals with no history of prior exposure to Salmonella either by vaccination or actual Salmonella infection had positive Widal tests. A number of studies have been performed in normal populations of different regions of the world to establish the background level of Salmonella agglutinins, in order to define a regional cutoff value for interpreting Widal test. Based on the previous study from the Kingdom of Saudi Arabia, a titer equal to or more than 1:160 was regarded as clinically significant. Because of the variable prevalence rates of Salmonella agglutinins, cutoff value for positive Widal test has been shown to exhibit a marked regional variation. A recent study on single acute phase sample from Kenya adopting a cutoff titer of 1:360 for both O and H antigens revealed that only 26% of patients had a diagnostic titer. Similarly, the majority of healthy individuals and those with various infections in the present study had reactive Widal test in titers less than 1:40 in contrast to another study from Kenya, where the majority reacted to titers either equal to or less than 1:80.

A significant number of patients suffering from other febrile illnesses in the present study were found to have a reactive Widal test and in some cases approaching clinical significance. However, a similar study performed on a smaller group of 46 patients with other febrile illnesses detected only one patient with a titer of 1:160 for O antigen. Salmonella organism is known for its ability to elicit cross reacting antibodies not only against O and H antigens, but also against other microbes as well. Generation of Salmonella agglutinins therefore may occur either by specific or by nonspecific stimuli. The detection of Salmonella in a statistically significant population of children suffering from malaria may indicate that nonspecific stimuli may trigger the formation of Salmonella agglutinins. Similarly, patients receiving treatment for carcinoma bladder with intravesical Bacillus Calmette-Guérin have been shown to exhibit high levels of Salmonella agglutinins in their sera, which returned to normal after the cessation of therapy. Reactive Widal test in a significant number of patients with other febrile disorders in this study could have been the result of microorganisms from unrelated species triggering the production of Salmonella agglutinins.

This study shows that compared with H antigen, O antigen was frequently detected at lower dilution but at the higher dilutions the observation was reversed. Although it is difficult to explain the diagnostic importance of this phenomenon, the titers of agglutinins against H antigen have been suggested to be a better indicator of Salmonella infection. Moreover, it has also been claimed that titers for any of the two antigens may reach diagnostic levels in the first two weeks of Salmonella infection. Further investigations are needed to gain a better understanding of the dynamics and behavior of O and H antigen titers in typhoidal and other febrile illnesses.

Widal test continues to be a subject of differing opinions with regards to its performance as a reliable diagnostic test for enteric fever. Among several issues, a higher percentage of false positive results are a major problem. Several febrile illnesses such as tuberculosis, malaria, and hepatitis B-associated...
polyarteritis nodosa have been shown to be associated with high incidence of false positive Widal test.[23–25] Detection of reactive Widal test in different clinical conditions other than enteric fever in the present study could either be the result of high levels of false positive Widal test in these conditions or the presence of cross-reacting antibodies, thus adding to the existing controversies.

Nested polymerase chain reaction (nPCR) has recently been shown to be a very sensitive and a specific tool for accurate diagnosis of enteric fever. Comparative analysis of the data shows that nPCR performs better than culturing the organism in patients with febrile illnesses of undetermined origin.[26] Similarly, immunoblotting has also been suggested to be a useful method for providing serological evidence of infection with Salmonella typhi, particularly for rapid diagnosis of typhoid fever.[27] The availability of such facilities however would still remain limited to specialized centers, and reliance on Widal test for diagnosis of enteric fever will probably continue until the introduction of a relatively simple, cost effective, and reliable test for detection of Salmonella infection.

Conclusion

Despite the associated problems, Widal test is commonly being applied in the laboratories for the diagnosis of enteric fever. A better understanding of the dynamics of Salmonella antigluttinins with regards to the background levels in an endemic area, along with the significance of O and H agglutinin titers in association with the clinical findings, is vital for a reliable diagnosis of enteric fever.

References


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