SIGNIFICANCE OF WIDAL TEST IN THE DIAGNOSIS OF TYPHOID FEVER

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ABSTRACT

Objective: The aim of our study was to determine the role of Widal test with appropriate titters in diagnosis of Enteric fever in an endemic area of District Swat.

Materials and Methods: The study was conducted in Saidu Teaching Hospital, Saidu Sharif between October 2010 and April 2012. Patients more than 15 years old and more than seven days of fever were included in this study. Widal test was also done on 200 normal individuals, 197 non-typhoidal fevers and 175 bacteriologically proven cases of typhoid.

Results: Of 200 normal individuals, 2% had an H agglutinin titre of 1/160 and 5% had an O agglutinin titre of 1/160. H and/or 0 agglutinin titre of 1/320 or more was observed in 93-97% of typhoid cases and in only 3% of patients with non-typhoidal fever. Of the sera from typhoid cases which gave a significant Widal reaction, the majority (79.9%) showed increases in both H and 0 agglutinins and 51 of 234 (21.8%) of these sera were collected in the first week of illness.

Conclusion: Based on these data it is evident that an H and/or 0 agglutinin titre of: 1/320 or more should be considered significant and indicative of typhoid fever.

Key words; Widal test, Gram negative bacterium, agglutinin titres

INTRODUCTION

Typhoid fever is a systemic infectious disease characterized by an acute illness, the first typical manifestations of which are fever, headache, abdominal pain, relative bradycardia, spleenomegaly and leukopenia[1,2]. Typhoid fever is caused by *Salmonella enterica* serotype Typhi, a Gram negative bacterium and is a major cause of morbidity and mortality worldwide. The common mode of infection is by ingestion of an infecting dose of the organism, usually through contaminated water or food. 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. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella enterica* serotype Typhi from the patient. Cultures of blood, stool, urine, rose spots, blood mononuclear cell, platelet fraction, bone marrow, and gastric and intestinal secretions can all be useful for diagnosis. Bacteria can be isolated from blood in 73 to 97% of cases before antibiotic use[2].

However, in the developing countries, facilities for isolation and culture are often not available especially in Swat.

Serodiagnosis of typhoid fever has been attempted since the late 19th century when Widal and Sicard showed that the serum of patients with typhoid fever agglutinated typhoid bacilli[3]. The resultant Widal test has been used very extensively in the serodiagnosis of typhoid fever and, in developing countries particularly remains the only practical test available. But 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 non-typhoidal fevers[4]. Unfortunately, neither the Widal test, which remains in widespread use in the developing world, nor any of the sero-diagnostic tests that have since been developed, has proven sufficiently sensitive, specific, and practical to be of value in areas where this disease is endemic[5]

The aim of our study was to find out the frequency of H and O agglutinin in normal healthy population

as well as in non-typhoidal illnesses and compare it with those having proven typhoid fever to enable us defining the cut off value of these titres in local population.

MATERIALS AND METHODS

This is a cross sectional observational study, that was conducted in the department of Medicine , Saidu Teaching Hospital ,Saidu Sharif, between October 2010 and April 2012. The study protocol was approved by Institutional ethical committee. Patients more than 15 years old who presented with more than 7 days of fever were included in this study . Patients who met the criteria were asked to give informed consent and answer a brief questionnaire about clinical signs and symptoms, antimicrobial treatment, and history of typhoid fever.

From all subjects, 5 ml of blood was obtained by venipuncture .Blood samples were centrifuged, and stored at -20°C, in order to minimize the degradation of antibodies in the serum, for Widal test. The test was performed by agglutination against the H and O antigen for Salmonella Typhi, paratyphi A and B.

The Widal slide agglutination test method was first used to screen subjects. Serum samples showing agglutination were then serially diluted with 0.85% saline from 1/40 to 1/1280 and subjected to tube dilution.

Salmonella enterica serotype Typhi was isolated from blood cultures by the BACTEC automated culture system (Becton Dickinson).

Patients were selected by non probability convenient sampling from Saidu Teaching Hospital whereas apparently healthy individuals aged 18 years and above were taken from students and staff members of Saidu Medical College and Jehan Zeb College Saidu Sharif.

All results were calculated and reported as percentages.

RESULTS

The level of H and 0 agglutinins in the normal population is presented in Table 1.

Table 1
(H and 0 agglutinins in 200 normal individuals)

Organism	Type of antigen	Agglutinin titres (no shown as %)				
		91/20	1/20	1/40	1/80	1/160
S.typhi	H	63	16	12	4	2
S.paratyphi A	H	77	7	7	5	2
S. paratyphi B	H	62	15	15	5	2
S.typhi	O	7	27	24	3	2
S.paratyphi A	O	91	6	1	32	5
S.paratyphi B	o	64	26	5	-	-
					2	-

For S typhi it can be seen that the majority (63%) had an H agglutinin titre of <1/20 with 2% having a titre of 1/160 .For 0 agglutinins, 32% had a titre of 1/80 and 5% had a titre of 1/160 (Table 1). Based on these data it was decided that an H and/or 0 agglutinin titre of 1/320 would be significant and indicative of typhoid fever. Using such criteria the levels of H and 0 agglutinins in bacteriologically proven typhoid cases and cases of non-typhoidal fever was determined (Table 2).

Table 2 Widal test results in normal individuals, cases of typhoid fever and non-typhoidal fevers

Group	**No in group**	**Widal test result***	
		Significant	Non significant
Normal	200	0	200(100%)
Typhoid fever	175	160(91.4%)	15 (8.5%)
Other fevers	197	6(3.0 %)	191 (97.0%)

*Significant resul/t: H and/or 0titre of(S typhi).
Non-significant result: H and/or 0 titre of-1/160 (v S typhi)

Results show that 91.4% of typhoid fever cases had a significant Widal test result. In contrast, only 3% of patients with non-typhoidal fever showed a significant Widal reaction. (Table 2).

Of the 15 typhoid fever cases who gave a non significant Widal test result, eight had been treated with full courses of antibiotics, two had concomitant infections and one was less than 10

years old. If these 11 cases were excluded from the group, a rate of 97% would be obtained for typhoid cases who gave a significant Widal reaction (Table 2).

DISCUSSION

Widal test has always been a subject of controversy in the contemporary medicine. But we in the developing world with enormous burden in the health care area and limited resources are always looking for cost effective measures especially investigations which are not very expensive.

It is generally recommended that Widal test should be interpreted in the light of base line titres in healthy local population of an endemic area. There have been numerous reports on the significance of a single Widal test but no consensus as to its diagnostic value in regions in which typhoid fever is endemic.[6-8]

In this study, an attempt has been made to assess the diagnostic value of the Widal test in an area where Typhoid fever is endemic by taking into consideration several factors which have led to confusion in the interpretation of the test. This study clearly show that in an endemic area such as swat, S typhi agglutinins against both H and 0 antigens may be present in the normal population at titres of up to 1/160.

It is clear that any interpretation as to the significance of a Widal test result must be made against this "baseline" information. For example, titres of 1/50 and 1/100 on a single specimen, which are considered significant in non-endemic areas [9], are of no diagnostic significance in areas where S typhi is endemic. On the basis of these findings we have set our own laboratory guidelines of H and/or 0 agglutinin titres of 1/320 as being of diagnostic significance. By using this criterion, a diagnostic titre of agglutinins was obtained in 93-97% of typhoid fever cases. A raised 0 agglutinin is of slightly greater diagnostic value than a raised H agglutinin. The data we obtained also does not support the conclusion that the titre for 0 antigen is the only useful value[9]. As a small proportion of patients with proven typhoid showed a rise only in H agglutinins. The results obtained are also of relevance to the notion that specimens which are taken in the first week of illness are of little use in the serodiagnosis of typhoid.

Studies have shown that false positive and negative results are more common in areas where non typhoidal salmonelosis is common[10]. Widal test may be falsely positive in persons who had previous vaccination or infection with salmonella typhi[10]. Raised Widal titres have also been reported in association with dysgamma-globulinemia of chronic active hepatitis and other autoimmune diseases[9-12]. On the other hand false negative results may be associated with early treatment, "hidden organism" in bones and joints, cases of relapses and infecting strains of poor antigenicity[9].

Another related issue frequently discussed is which of the two antigens are more significant, i.e. O and H. There are some conflicting reports in literature but our study was in favor of the significant rise of both titres together in proven cases.

There have been several studies conducted in Pakistan and in neighboring countries addressing the same issue. Aftab and Khurdhid from Lahore have reported a similar study concluding that elevated level of H and O agglutinin titer of 1:320 is of diagnostic value in our setup[10]. Sherwal et al have published a comparative study from India[11] whearas Alam et al have data from Bangladesh supporting the same[12]. Khoharo from Sind have published data from Mirpur Khas on 50 patients evaluating single acute phase widal test advocating titers of 1:320[13].

Despite all this data and the new developments in this field, the diagnosis of typhoid in much of the developing world is made on clinical criteria[14-16]. It is therefore of utmost importance that the scope and role of Widal test must be clearly defined with all its limitations to the general health care professionals[17-18].

CONCLUSION

We thus conclude that in endemic areas the Widal test is still of significant diagnostic value provided judicious interpretation of the test is made against a background of pertinent clinical information, and epidemiological data which relate to agglutinin levels in normal individuals and in non-typhoid fevers common in the region. We also point out that in endemic areas a single Widal test can be of diagnostic value in the early stage of disease and thus help in reducing morbidity and mortality from typhoid.

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