The Widal test in the diagnosis of typhoid fever in the Transvaal


Summary

We analysed the results of the Widal test in the northern and eastern Transvaal in relation to bacteriologically confirmed cases of typhoid fever, patients suspected of having the disease, febrile patients without typhoid fever and healthy individuals.

Titres of 1:200 or greater for either H or O agglutinins were recorded for 75.2% of patients with bacteriologically proven typhoid fever, 4.6% of healthy subjects residing in an endemic area and 7.5% of patients presenting with non-typhoid fevers. Age, sex and region were found to affect the percentage of positive tests recorded. Despite these failings, the Widal test was found to be of value in the diagnosis of typhoid fever. The concept of a diagnostic titre was considered unreliable, but considered in conjunction with the clinical picture, O or H agglutinin titres of 1:200 or more may be regarded as strong presumptive evidence of typhoid fever.


The Widal test has been in use as an aid in the diagnosis of typhoid fever since the beginning of this century. Its use has been both praised and condemned. The test is based on demonstrating agglutinating antibodies (agglutinins) in the serum of an infected patient, against the H (flagellar) antigen (d) and the O (somatic) antigen complex (1, 9, 12) present in the causative organism Salmonella typhi.

The definitive diagnosis of typhoid fever depends on the isolation of S. typhi from the blood, stools, urine or other body fluids. The role of the Widal test is primarily to demonstrate a fourfold rise in the agglutinin titre during the illness.

In the northern and eastern Transvaal typhoid fever is still a common disease among the Black population — the annual rate of notification is 2 - 3 times as high as the corresponding rate for the RSA as a whole. The incidence rates for Venda, Kangwane and Venda authorities during 1977. In some areas, owing to the establishment of densely populated villages without adequate water supplies and sewage disposal facilities, the incidence of the disease has increased. In many rural areas transportation of blood and faecal samples for bacteriological diagnosis to distant laboratories is a problem and the submission of blood for serological tests at the expense of a definitive bacteriological diagnosis is often preferred by clinicians. Laboratory facilities are limited and basic tests have had to be restricted because of escalating costs. Bacteriological diagnosis is therefore seldom attained. We wish to show that the Widal test is still useful in the diagnosis of typhoid in this area.

Patients and methods

The Widal test was performed on 4 different groups of patients in the Transvaal.

Group A

A random selection of 74 households was taken from Rita village, Lebowa. From a population of 647 people, 204 individuals were tested, of whom 46 were aged 0 - 5 years, 47 aged 6 - 10 years, 45 aged 11 - 15 years, 18 aged 16 - 20 years, 8 aged 21 - 25 years and 40 were over 26 years. A further 78 individuals, labourers on a neighbouring citrus estate, were also tested. Their ages ranged from 18 to 60 years. When tested they had no complaints of illness and as far as could be ascertained they had not been immunized during the preceding 2 years. The Widal tests were performed by the South African Institute for Medical Research at Pietersburg and Duiwelskloof. The Institute's own standard agglutinating H and O suspensions, prepared by the central laboratory in Johannesburg, were used.

Group B

This group consisted of 67 patients presenting with non-typhoid febrile illnesses at Letaba Hospital, Gazankulu, 15 km from Rita village. These patients were seen during the months April - August 1979. Widal tests were performed on at least two occasions, a minimum of 6 days apart, in the laboratory of the National Institute for Tropical Diseases, Tzaneen. Agglutinating suspensions manufactured by Wellcome Laboratories were used.

Group C

This group consisted of 330 patients with bacteriologically proven typhoid fever. Of these, 275 presented at Themba Hospital between December 1974 and December 1978. The Widal test was performed either at Themba Hospital laboratory, using Wellcome reagents, or at the SAIMR laboratory at Nelspruit, using the Institute's own reagents. The test was performed on admission and repeated again after 7 - 10 days in most cases. Only the highest agglutinin titre was recorded in the results. The remaining 55 patients presented at the other hospitals (listed under group D) and the Widal test was done on admission only.
Group D
The final group consisted of 763 patients suspected clinically of having typhoid fever. Presentation was at various hospitals in the northern Transvaal (Themba Hospital near Nelspruit, Donald Fraser Hospital in Venda, Elim Hospital near Pietersburg, Blouburg and Helene Franz Hospitals in Lebowa and Pietersburg Hospital) between December 1974 and March 1979. The Widal test was performed at the SAIMR laboratories at Pietersburg and Nelspruit and the hospital laboratories at Themba and Elim. In the majority of these patients only one Widal test was done on admission. Where the test was repeated, only the highest titre is recorded in the results. This group included the 330 patients studied under group C.

Results
Variations in typhoid agglutination results may occur when different techniques and reagents are used. No attempt was made to correct possible discrepancies in the results between the different laboratories, but analysis of the Widal test on 20 serum samples submitted to the laboratories involved in this study, revealed that the correct diagnosis, based on a titre ≥ 200 for H or O agglutinins, would have been obtained in 85% of the patients.

Group A
The agglutinin titres for the 282 healthy individuals are represented in histogram form in Fig. 1.

One patient had been vaccinated within the last year. The figure of 95.4% of healthy individuals showing a titre of < 1:200 would increase to 97% should those patients with active typhoid fever be excluded from the total.

According to Collard et al., any titre that occurs in more than 5% of the normal population can be regarded as not a significant indicator of active infection. In this group the titre of ≥ 1:200 in less than 5% of the normal population is a significant indication of active infection.

Group B
The likely illnesses responsible for the pyrexia in the 67 patients (31 males and 36 females) who presented at Letaba Hospital but were not suffering from typhoid fever are summarized in Table 1. Of the total of 67 patients, 9 (14.5%) showed some agglutinin titre and 5 (7.5%) showed a titre ≥ 1:200 for H and O agglutinins. None of the 0-4-year group had detectable titres. If this group is excluded, 9 (20%) sera from the remaining 45 patients showed agglutination reactions and 5 (11%) had a titre ≥ 1:200 for H and O agglutinins. Of these 5 patients, 2 (a 35-year-old woman and a 12-year-old boy) had respiratory infections with H agglutinin titres of 1:200. The other 3 patients (a 24-year-old woman, a 12-year-old girl and an 8-year-old boy) all had, in addition to their febrile illnesses, an underlying chronic liver disorder with enlarged spleen. One had persistent titres of 1:400 and the other 2 persistent titres of 1:1 600 (both H and O). Of these 5 patients, 2 had persistent titres of 1:1600 (both H and O) over a period of months. They both had urinary schistosomiasis and on many occasions cultures were negative for S. typhi.

Table 1. Diagnoses in non-typhoid febrile illnesses

<table>
<thead>
<tr>
<th>Age group (yrs)</th>
<th>No. of patients</th>
<th>Diagnosis (No. of patients in parenthesis)</th>
</tr>
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<tbody>
<tr>
<td>0 - 4</td>
<td>Male Female (No. of patients in parenthesis)</td>
<td></td>
</tr>
<tr>
<td>13 9</td>
<td>Measles and complications (15) Respiratory infections, including pneumonia (4) Gastro-enteritis, encephalitis, meningitis (1 each) (4 patients had severe malnutrition) Respiratory infections, including pneumonia (5) Minor fevers, probably viral (3) Malaria, measles, cellulitis (2 each) TB arthritis, chronic hepatitis, schistosomiasis, TB pleurisy, gastro-enteritis, cerebral tumour, chronic diarrhoea with hepatosplenomegaly and pellagra (1 each) (4 patients had severe malnutrition)</td>
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<tr>
<td>5 - 14</td>
<td>11 11</td>
<td>Respiratory infections, including pneumonia (5) Minor fevers, probably viral (3) Malaria, measles, cellulitis (2 each) TB arthritis, chronic hepatitis, schistosomiasis, TB pleurisy, gastro-enteritis, cerebral tumour, chronic diarrhoea with hepatosplenomegaly and pellagra (1 each) (4 patients had severe malnutrition)</td>
</tr>
<tr>
<td>15 - 24</td>
<td>3 10</td>
<td>Respiratory infections, including pneumonia (4) Pyrexia of unknown origin (2) Urinary tract infection (2) Chronic hepatitis, pulmonary TB, measles, septic vulvitis and gastro-enteritis (1 each)</td>
</tr>
<tr>
<td>25 and over</td>
<td>4 6</td>
<td>Respiratory infections (4) Urinary tract infection in pregnancy (1) Anaemia due to rectal bleeding (1) Hepatitis, malaria, pyrexia of unknown origin, pelvic abscess (1 each)</td>
</tr>
</tbody>
</table>

Fig. 1. Agglutinin titres in 282 sera from healthy subjects in an endemic area.
Group C
This group consisted of 330 patients with bacteriologically proven typhoid fever. The agglutinin titres obtained are shown in Fig. 2. Agglutinin titres $\geq 1:200$ were found in 64 (19.4%) patients for H agglutinins only, in 50 (15.2%) for O agglutinins and in 134 (40.6%) for both H and O agglutinins. Of the 330 patients, 248 (75.2%) had agglutinin titres $\geq 1:200$ and 30 (9%) had no demonstrable agglutinin titre at any stage. Rising titres were often demonstrated among the 275 Themba Hospital patients, but in only 18 (6.5%) of the cases did the titre in the second specimen become elevated to 1:200 or more from an initial lower titre.

Fig. 2. Agglutinin titres in 330 sera from patients with bacteriologically proven typhoid fever.

Group D
A total of 763 patients (331 males and 432 females) presented with suspected typhoid fever. The agglutinin titres obtained are shown in Figs 3 and 4.

Titres $\geq 1:200$ were recorded for 75% of the patients. The H agglutinin and the male patients showed an overall higher percentage of titres $\geq 1:200$. If the percentage of suspected typhoid patients with titres $\geq 1:200$ is related to the hospitals at which they presented it can be seen that the percentages vary from region to region (Table II).

The results for 470 of the suspected typhoid patients were related to age; 50 (4%) were aged between 6 and 15 years.

Fig. 3. Agglutinin titres in 331 sera from male patients with suspected typhoid fever.

Between birth and 5 years the O agglutinin titre was $\geq 1:200$ in 60% and the H agglutinin titre $\geq 1:200$ in 42% of cases. This pattern reversed with age, and in patients over 25 years of age the H agglutinin titre was $\geq 1:200$ in 60% and the O agglutinin titre $\geq 1:200$ in 48.5% of cases.

Discussion
In the typhoid-endemic areas of the northern and eastern Transvaal, the definitive diagnosis of typhoid fever can be a problem to clinicians because as a clinical entity it differs in many respects from the description in textbooks based mainly on experience in developed countries. The protean features which may be encountered have been well described by Wicks and others in Rhodesia. The traditional views that agglutinin titres only become positive towards the end of the second week of the illness, rising through the third week, and that H agglutinin titres are of very limited value, do not appear to be generally true in this region.

Wicks et al. and Senewiratne both found that high agglutinin titres could be demonstrated at an early stage in the illness, often during the first week. This suggests that, in an endemic area with frequent exposure to S. typhi and antigenically related salmonellae, the immune response may often not be a primary one.

Even though patients tend to come to hospital late in the course of their illness, it was frequently noted that those who appeared during the first week had significantly elevated titres; thus, a single Widal test was initially positive in 70% of cases. This probably accounts for the finding that a fourfold rising titre was so rarely demonstrated. The most common finding was a two- or threefold rise. Only 6.5% of the patients had initial titres $< 1:200$ which became elevated later. The effect of chloramphenicol in depressing the antibody response undoubtedly contributed to this low figure.
The H agglutinin titre in general was positive more often than the O agglutinin titre. This is in agreement with the findings of Wicks et al. and also of Brodie, who showed that H agglutinin titres ‘provided a more reliable aid to diagnosis’ than did the O agglutinin titres. In contrast, Schroeder states the traditional view that ‘because the H antigen titre is extremely variable and can rise as a non-specific response to other infections, it is of little value in diagnosing typhoid fever’.

The findings among the 282 healthy individuals showed, firstly, that few residual agglutinin levels were found and, secondly, that a titre of H or O of ≥ 1:200 was a significant indication of active typhoid fever among people living in this highly endemic region. It is also suggested that mild or subclinical typhoid infection occurs frequently and is an important part of the reservoir of organisms in the endemic areas.

In the series of non-typhoidal febrile illnesses it was found that 11% of patients over 4 years of age had titres ≥ 1:200. In 2 patients titres were persistently 1:160, suggesting a possible active infection which could not be confirmed despite repeated culture attempts. There was no history of a recent infection or of immunization. It is more likely that this situation was associated with a hepatic lesion or a schistosomal infection. It is suggested that these findings show the danger of thinking in terms of ‘diagnostic’ titres: the extremely high titres may occur in the absence of active infection with S. typhi.

In this situation, the only conclusive proof of typhoid fever is a rising titre of agglutinins and the exclusion of infection by any other salmonellae.

The finding of a titre ≥ 1:200 in 75% of the bacteriologically proven cases compares with figures of 76% shown by Sen and Sasena and 94.3% shown by Senewiratne, taking a titre of 1:160 as ‘positive’. Using a titre of 1:80, Wicks et al. found 93% ‘positive’ in Salisbury. The lower titres considered as ‘positive’ may account for the difference between their results and ours. In 9% of the bacteriologically proven cases, no agglutinins were demonstrated. This correlates with the generally accepted figure of 10%. The differences discovered on analysing the large number of suspected typhoid patients in relation to the different hospitals in the region were surprisingly large and not readily explained. Perhaps some hospitals had a higher diagnostic accuracy or were more selective in performing this investigation.

The differences noted for age and sex are interesting: probably the greater reliability of the H agglutinin titre with increasing age is a reflection of the longer exposure to infection and immunization in an endemic area.

A number of aspects should be kept in mind as possible causes of error or confusion in the interpretation of the Widal test: 1. The possibility of cross-reaction with antibodies induced by organisms other than S. typhi must be considered. Several other salmonellae share H and O antigens with S. typhi. A rise in titre of these antibodies need not, therefore, be specific for S. typhi. The prevalence of infections with these organisms in the population should be determined.

2. Immunoglobulins found in certain immunologically abnormal states may cause cross-reactions. Senewiratne studied 61 patients with various diseases characterized by major immunological disorders, including rheumatoid arthritis, rheumatic fever, the nephrotic syndrome, ulcerative colitis, multiple myeloma and other collagenoses, and found H and/or O antibody titres ≥ 1:160 in 11.5%. Patients with chronic active liver disease are reported to have raised antibody titres. Similarly, narcotic addicts have higher titres than expected. In schistosomiasis, which is also endemic in this region, higher antibody levels have been found, especially in patients with portal hypertension and splenomegaly.

3. The significance of an antibody titre should be decided upon only in relation to the titres found in the population of the area with non-typhoidal fevers, as an anamnestic reaction (nonspecific rise in titre) may occur. In an endemic area it would be expected that the ‘resting’ or ‘background’ antibody titre will be higher than among a population from a non-endemic area. The extent of this should be determined for the population of the specified region, as has been done in this article.

4. Antibody levels after vaccination with TAB or typhoid vaccine would be higher, affecting the diagnostic value of the test. In some areas of the northern Transvaal large-scale vaccination has been practised periodically, and this must be kept in mind.

5. Salmonella agglutinins may not be produced at all. This situation has been reported as occurring in about 10% of cases. There may be an associated abnormality such as severe hypoproteinaemia, but usually no obvious reason is found. The organism may be in a site inaccessible to the antibody-producing system, such as a joint. It has been shown that chloramphenicol depresses immunoglobulin synthesis, and this may prevent rising titres of antibody being demonstrated.

6. It should be appreciated that laboratory technique is of vital importance in performing any immunological test. The standard reagents need to be carefully controlled and scrupulous attention to detail must be given by technicians. It has been shown how often inaccuracy can be responsible for discrepancies, and to some extent this has detracted from the usefulness of this test. In this article the results from different laboratories have been taken in order to guide the physician practising in this area in interpreting results forwarded by the local laboratory.

Conclusion

The Widal test still has an important role to play in the diagnosis of typhoid fever in this region. A titre of ≥ 1:200 of either H or O agglutinin in a patient suspected on clinical grounds of having typhoid fever is a significant indicator of active infection, but a rising titre must be looked for. Apart from bacteriological isolation of S. typhi (which also is not foolproof, since a carrier state may exist) a fourfold rising agglutinin titre is the most definitive evidence for a current attack of typhoid fever. Blood cultures should always be examined early in the disease and the Widal test must be repeated after a period of at least 7 - 10 days. The effect of chloramphenicol on depressing the antibody titre must also be borne in mind. In the future more reliable and specific serological tests will be developed as well as improved vaccines, and these factors will alter the present status of the Widal test. Until then, and for the foreseeable future, this test, if correctly interpreted, can be a useful aid to the diagnosis of typhoid fever.

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