THE VALUE OF THE WIDAL TEST IN THE DIAGNOSIS OF PROLONGED SEPTICEMIC SALMONELLOSIS (1)

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SUMMARY

Twenty patients with prolonged septicemic salmonellosis (Group 1) and 20 with schistosomiasis mansoni (Group 2) were selected for this study. In both groups, the Widal test was done using antigens of the sample Ty 901 (S. typhi). The test was also applied in 6 group 1 patients with antigens prepared from salmonellae isolated from these patients (autoantigens). Titres over 1:200 were considered significant. Ten group 1 patients (50%) were positive for antigen “H” and 5 (25%) were positive for antigen “O”. Three patients with negative “H” and “O” reactions became positive with high titres when using autoantigens. Two other cases maintained the same positive titres and one case showed a fourfold increase in titres when the test was done with antigens of the Salmonella isolated. The Widal test was positive in most patients infected with group D Salmonellae. Considering titres above 1:200, all cases were negative in Group 2. The authors conclude that the Widal test has low positivity in prolonged septicemic salmonellosis. The test may be valuable in the diagnosis of this disease when using S. paratyphi “A” and “B” antigens and a mixture of Salmonella antigens taken from other groups.

INTRODUCTION

The value of the Widal test in the diagnosis of Prolonged Septicemic Salmonellosis (PSS) is not adequately defined. A few studies (1) have shown wide variations in agglutinin titres, with a significant number of patients presenting negative or inconclusive results. TEIXEIRA (2) states that the Widal test is positive in many cases when S. typhi and S. paratyphi are the causal agents. NEVES & MARTINS (3) report many negative results, even when S. typhi is identified in blood cultures. Various suggestions to explain negative test results were made: a) anti-O agglutinin titres might fall with duration of disease; b) a possible “O” blocking effect may occur in cases with Vi agglutinin; c) patients with disease caused by Salmonellae belonging to other groups (not S. typhi or S. paratyphi, on which the Widal test is based) might not produce antibodies capable of agglutinating classic Widal test antigens; and d) some patients might not produce anti-Salmonella antibodies due to immunologic failure of the B lymphocyte system.

This paper presents the results of a prospective study on the positivity of the Widal test with classic antigens and with autoantigens (prepared from Salmonellae isolated from each patient) in PSS.

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MATERIAL AND METHODS

Identification of patients

Group 1 — twenty patients with PSS whom were admitted to the Carlos Chagas Hospital (Medical School of Federal University of Minas Gerais, Brazil) between 1972 to 1982 were selected. The diagnosis of the disease was based on 3 criteria: a) fever with over 3 weeks duration; b) the presence of Salmonella sp in at least two blood cultures; c) viable S. mansoni ova in stool examinations. The patients were 5 to 20 years of age (mean 12.7), 11 male, 9 white and 11 non-white.

Group 2 — twenty patients with schistosomiasis mansoni were selected as controls. Fifteen cases were diagnosed by clinical and epidemiologic histories and positive stool examinations for S. mansoni ova. One case was diagnosed by a positive rectal biopsy and the remaining 4 by liver biopsies. All cases had the hepatesplenic form of the disease. Patients were 9 to 46 years of age (mean: 21.7), 13 male, 13 white and 7 non-white.

Patients in both groups had not been vaccinated against typhoid fever and also denied previous history of typhoid infection.

Isolation and identification of Salmonellae

The following procedure was used for the isolation of salmonellae in the Laboratories of the School of Medicine: a) 10 ml of blood were added to 100 ml of BHI (Brain Heart Infusion) broth and kept at 37°C, according to BIER; b) the broth was observed up to 30 days, and when turbidity developed, transfer was carried out to blood agar and Teague media. After 24 hours in the former, a smear was stained by Gram's technique, and whenever Gram negative rods were found, colonies were removed to TSI agar media. The same procedure was applied to Teague cultures; c) if suspected salmonellae were observed, the usual biochemical tests were applied; d) organisms with biochemical results characteristic of salmonellae were submitted to agglutination tests with polyvalent somatic and flagellar anti-salmonella serum. After this procedure, the cultures were transferred to Lignieres media and sent to the Oswaldo Cruz Institute (Rio de Janeiro) for serological typing.

Blood cultures were made for all group 1 and 15 group 2 patients.

Widal test with classic and autoantigens

The Widal test with classic antigens was performed for all group 1 and 2 patients, and the reaction with autoantigens was applied in 6 group 1 patients.

Antigens and dilutions

Somatic (O) and flagellar (H) antigens were prepared with Ty 901 samples for detection of anti-salmonella typhi antibodies. The same method was used for producing antigens with samples taken from patients with PSS (autoantigens) to check antibody titres related to serotypes isolated from each patient.

Agglutinin titres were determined with serum serial dilutions (1:10 to 1:1280), with antigens added as described in the classical method used by Widal. Titres over 1:200 were considered significant.

Statistical analysis

The chi-square ($X^2$) test with Yates' correction was used to 5% significance level.

RESULTS

The Widal test with “H” and “O” antigens was negative in 7 (35%) group 1 patients. In 13 cases, the test was positive for antigen “H” and in 12, it was positive for antigen “O”. Considering titres over 1:200 as significant, 10 patients (50%) were positive for antigen “H” and 5 (25%) for antigen “O”. The Widal test was positive in all patients from which S. typhi was isolated (Table I).

The Widal test was negative in 12 group 2 (control) patients (60%). This difference is not significant when compared to group 1 ($P > 0.05$). Considering titres over 1:200 as positive, the reaction was negative in all cases.

Six group 1 patients were tested with autoantigens (cases 2, 6, 7, 9, 12 and 13). Sera of three patients initially non-reacting with “H” and “O” antigens were positive with high titres when using autoantigens. The remaining 3 cases (cases 9, 12 and 13) had positive Widal tests, and two of them maintained titres after
autoantigen testing. Case 9 (S. kentucky) presented a fourfold increase in agglutinin titres when tested with Salmonella autoantigens.

From 11 patients infected with Group D Salmonellae only one had a negative Widal test. On the other hand, the same test was negative or presented low titres in patients infected with group A, B and C Salmonellae (Table I).

**DISCUSSION**

We conclude that the Widal test is of little value in the diagnosis of PSS. Significant diagnostic agglutinin titres were found in 25% for “O” and in 50% for “H” antigens. As antigen “H” is less important due to anamnestic reactions with other infections, the serologic diagnosis of PSS becomes restricted. The duration of the disease does not seem to influence positivity, as pointed out by TEIXEIRA. We found cases with high agglutinin titres at both extremes (Table I).

Negative tests converting to positive when using autoantigens are in agreement with predictions made by NEVES & MARTINS. The positivity of the Widal test in patients infected with Group D Salmonellae is explained by the presence of common antigens in Salmonellae of the same group of S. typhi. The disease caused by Salmonellae of other groups shows negative Widal tests. Positive results in the four S. typhi cases confirm previous observations; it must be noted that 3 of these patients had anti-O agglutinin titres under 1:200. In only one case was S. paratyphi A isolated and the Widal test was negative. Using S. paratyphi A antigens, the test would probably become positive. In case 20, Group D S. berta was isolated, and the test was negative. Further explanations are needed in these cases, as both S. typhi and S. berta have common determinant antigens (antigens 9 and 12).

The Widal test was positive in 8 group 2 (control) patients (40%), with titres under 1:200. Similar results were obtained by SHIKANAI-YASUDA et al. in patients with hepato-splenic schistosomiasis in the same age groups.

It may be concluded that the Widal test with “H” and “O” antigens yields few positive results in patients with PSS. Using S. paratyphi A and B antigens and a selection of antigens of Salmonellae belonging to various groups (determined by the local prevalence of Salmonellae, Table I), we found cases with high agglutinin titres at both extremes.
Ilae), positivity may be increased, enabling the Widal test to become a useful tool in the diagnosis of this disease.

RESUMO

Valor da reação de Widal no diagnóstico da Salmonelose Septicêmica Prolongada (SSP)

Vinte pacientes com SSP (Grupo 1) e 20 com esquistossomose mansoni (Grupo 2) foram selecionados para o estudo. Realizou-se a reação de Widal, com antígenos preparados da amostra Ty 901 (S. typhi), nos pacientes dos Grupos 1 e 2. Em seis pacientes do Grupo 1, a reação de Widal foi feita com antígenos preparados a partir de salmonelas isoladas destes pacientes (autoantígenos). Foram considerados significativos os títulos superiores a 1:200. Dez pacientes do Grupo 1 (50%) tiveram a reação positiva para o antígeno “H” e cinco (25%) para o “O”. Em quatro casos em que se isolou a S. typhi, a reação de Widal foi positiva. Em três pacientes com reação negativa para “H” e “O”, houve viragem da reação com títulos elevados quando se utilizou autoantígenos; outros dois casos positivos mantiveram os mesmos títulos e em um caso houve elevação de quatro vezes nos títulos quando a reação foi feita com antígenos da Salmonella isolada. A reação de Widal foi positiva na maioria dos casos quando as salmonelas isoladas eram do Grupo D. Para o grupo 2, caso se aceitem os títulos superiores a 1:200 todos os exames foram negativos. Os Autores concluem que a reação de Widal apresenta baixa positividade na SSP. Com o uso de antígenos de S. paratyphi A e B e de uma mistura de antígenos de salmonelas de grupos distintos, a reação provavelmente será de valor no diagnóstico da SSP.

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