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Full Length Research Paper

Widal test case study in Togo: Proposition for a rational use

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The Widal test is still being used in Togo and the results are labelled "positive" even for only one agglutinin without titration. As a result of that, more and more people claim to have typhoid fever while the clinical state is not suggestive and no bacteriological test is performed. We discuss here the results of 200 patients seen in routine laboratories from November 2005 to April 2006. The agglutinin TO, TH, AO, AH, BO, BH, CO, CH were tested in tube and the titre determined for any positive agglutination. We have put together the result of all the agglutinins to have a complete serological profile toward the pathologic *Salmonella*. Malaria test was performed and the clinical status of the patient checked. Only 3 patients (1.5%) had the serological profile of an infection to *Salmonella typhi*, 154 patients had a clear negative profile and 42 patients had an intermediate profile. The 42 intermediate profiles are subjects to interpretation. The various hypotheses to explore were a serological scar of an old infection, a headed infection due to an early antibiotherapy, a cross reaction to *Salmonella* with another germ having a common antigen O. When the patient was positive to malaria test, the most probable disease was malaria in endemic area because of the cross reactivity. The rate of positive malaria was 100% among the 3 patients with positive profile in Widal test versus 3.9% among the 154 patients negative in Widal test and 26.2% among the 42 patients with intermediate serological profiles. The number of positive results is small, pointing out the weakness of clinical diagnosis preceeding the Widal test. The number of intermediate results is high, raising the need of adequate interpretation and labelling of the Widal test in order to reduce wrong interpretation.

Key words: Widal and Felix, typhoid, *Salmonella*, serology.

INTRODUCTION

Typhoid fever is still a public health problem in tropical countries with low level of hygiene (Hamze and Vincent, 2004; Hamze et al., 1998). The disease is endemic in Africa, South East Asia, Central America and South America (Hamze and Vincent, 2004; Parry et al., 1999). In Togo, the obsession of typhoid fever leads professionals and patients to request a Widal test. Patients are seen once and the result is labelled "positive", even when there was only one agglutination. Some laboratories mention the specific agglutinins that are positive and their titre but even then, practitioners assume the patient to have typhoid fever because of misunderstanding of the word "positive" there. The situation is worsened by an early antibiotic medication which inhibits the classic production of antibodies and makes it difficult to monitor the kinetics of agglutinins (Parry et al., 1999; Olopoenia, 2000; Rodrigues, 2003; Avril et al., 1988; Saha et al., 1996). This leads to a non rational use of antibiotics active on *Salmonella typhi* such as chloramphenicol and ciprofloxacine (Hamze and Vincent, 2004; Hamze et al.,

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1998; Parry et al., 1999; Shukla et al., 1997; Abraham et al., 1981). In this situation, more than 30% are assumed to have a typhoid fever in some hospitals of Togo (Agbenu et al., 2010). From some results obtained in routine practice, we propose here comments on serological profiles.

MATERIALS AND METHODS

Sera obtained from 200 patients were analysed with the kit *SALMONELLA* SEROLOGY / BIO-RAD®. We performed the Widal test in tube with centrifugation according to the operating manual. If agglutination occurs with any antigen, we did successive dilutions to determine the titre (Agbenu et al., 2010). The antigens tested were TO, TH, AO, AH, BO, BH, CO, CH. The titre was given by the last dilution which agglutinated. A patient was assumed seropositive if he had the following agglutinins:

1) H at least at 400 if alone,
2) H at 200 and O at 200 at least when both were present.

A patient was seronegative if the titre of each O and H agglutinins is lower than 200. Other cases were intermediate. The agglutinins were lower and their profiles did not correspond to any of the major *salmonella* (*S. typhi, paratyphi* A, B and C). We proposed hypotheses to explain the intermediate serological profiles. All the patients were tested for malaria by microscopy, thick smear with May Grünwald Giemsa staining. Epidemiological data (age, sex, location, occupation…) and clinical data (fever, asthenia, digestive signs, neurological signs…) were also collected.

RESULTS

Over 200 patients who came to the laboratories with the request of a Widal test, 3 had a positive serology to *S. typhi* and 42 were neither positive nor negative to any of the *Salmonella* antigen typhi, paratyphi A, B or C. The corresponding serological profiles are presented in Table 1. The intermediate results should have generated some hypotheses that are compiled in the discussion. Fever was present in 100 patients (50.0%), asthenia in 111 (55.5%), stomach-ache in 79 (39.5%), headache in 45 (22.5%), nausea and vomiting in 22 (11.0%), anorexia in 49 (24.5%) and diarrhoea in 20 (10.0%). Some patients had association of symptoms. The three patients, positive with Widal test, had asthenia (2 cases), fever (2 cases), stomach-ache (3 cases), diarrhea (2 cases), nausea and vomiting (1 case), headache (2 cases) and vertigo (1 case).

Abdominal pain ($p<0.034$) and diarrhea ($p<0.008$) were more frequent in positive than in negative cases. No significant difference was noted in the occurrence of symptoms between the positive and the intermediate cases. Headache ($p<0.004$), abdominal pain ($p<0.000$), diarrhea ($p<0.026$) and anorexia ($p<0.0198$) were more frequent in intermediate than negative cases. Fever was more frequent in negative than intermediate cases ($p<0.041$).

DISCUSSION

In many routine laboratories, these intermediate serological profiles (21.0%) were wrongly labelled “positive” and based on that, some practitioners initiated a treatment. The aforestated results show that all occurrence of agglutination cannot be assumed as an occurring “typhoid fever”. The laboratories should add an interpretation of the whole serological profile and relate it to the patient’s clinical condition. Moreover, the test has to be repeated in order to monitor the kinetics of agglutinins. Irrespective of the diagnostic value of the Widal test in endemic regions, the intermediate serological profiles presented earlier could be interpreted with the hypotheses stated subsequently:

Hypotheses 1 and 2: Early Typhoid fever (8th day), or infection by a *Salmonella* which shares antigen O with *S. typhi* with a different antigen H.

Hypothesis 3: These patients appeared to have a serological scar of a past typhoid fever. It could be an infection due to non-thyphoidal *Salmonella* which shares the antigen H with *S. typhi* with a different antigen O. The patients were positive to malaria test.

Hypothesis 4: The patient appeared to have a serological scar of a past typhoid fever. It could be an infection to non-thyphoidal *Salmonella* sharing the antigen H with *S. typhi* but a different O antigen. The patients were positive to malaria test.

Hypothesis 5: It could be an early paratyphoid A infection with coagglutination with BO, or an infection due to a *Salmonella* sharing the O antigen with *S. paratyphi* A. The patient was positive to malaria test.

Hypothesis 6: The patient appeared to have a serological scar of a past typhoid fever, or an infection to non-thyphoidal *Salmonella* sharing the antigen H with *S. typhi* but a different O antigen. The patient was positive to malaria test.

Hypothesis 7: The patient appeared to have a serological scar of a past paratyphoid B fever, or an infection to non-thyphoidal *Salmonella* sharing the antigen H with *S. paratyphi* B. The patient was negative to malaria test.

Hypothesis 8: The patient appeared to have an early paratyphoid B fever with a coagglutination TO or an infection to non-typhoidal *Salmonella* sharing the antigen O with paratyphi B, but a different H antigen. The patient was positive to malaria test.

Table 1. Serological profiles towards *Salmonella typhi* and *paratyphi* in 200 patients who underwent Widal test in Togo.

Titre of agglutinin								MS	Serological profile	Number (%)
TO	TH	AO	AH	BO	BH	CO	CH			
0	0	0	0	0	0	0	0	Neg	Neg	154 (77.0)
200	0	0	0	0	0	0	0	Pos	Hypothesis* (1)	9 (4.5)
200	0	0	0	0	0	0	0	Neg	Hypothesis* (2)	1 (0.5)
0	100	0	0	0	0	0	0	Neg	Neg	5 (2.5)
0	200	0	0	0	0	0	0	Neg	Hypothesis* (3)	9 (4.5)
0	400	0	0	0	0	0	0	Pos	Typhoid fever treated early	2 (1.0)
100	200	0	0	0	0	0	0	Pos	Hypothesis* (4)	2 (1.0)
400	800	0	0	0	0	0	0	Pos	Typhoid fever	2 (1.0)
400	800	0	100	0	0	0	0	Pos	Typhoid Fever	1 (0.5)
0	0	100	0	0	0	0	0	Neg	Neg	3 (1.5)
0	0	100	0	100	0	0	0	Pos	Neg	1 (0.5)
0	0	200	0	100	0	0	0	Pos	Hypothesis* (5)	1 (0.5)
0	200	0	100	0	0	0	0	Pos	Hypothesis* (6)	1 (0.5)
0	0	0	0	100	0	0	0	Neg	Neg	5 (2.5)
100	0	0	0	100	200	0	0	Pos	Hypothesis* (7)	1 (0.5)
100	0	0	0	200	0	0	0	Pos	Hypothesis* (8)	1 (0.5)
0	200	200	0	100	0	0	0	Pos	Hypothesis* (9)	1 (0.5)
0	200	0	0	200	0	0	0	Pos	Hypothesis* (10)	1 (0.5)

* See discussion, Neg= negative, Pos= positive , MS= malaria smear.

Hypothesis 9: The patient appeared to have a serological scar of a past typhoid fever on which occurs an early paratyphoid A fever with coagglutination, or an infection due to non-typhoidal *Salmonella* sharing the antigen O with *S. paratyphi* A but a different H antigen. The patient was positive to malaria test.

Hypothesis 10: The patient appeared to have a serological scar of a past typhoid fever on which was occurring an infection due to *Salmonella* sharing the antigen O with *S. paratyphi* B but a different H antigen. The patient was positive to malaria test.

False positive reactions can occur during some diseases: malaria, exanthematic typhus, dysglobulinemia (myeloma, collagenosis, cirrhosis) and various infections by enterobacteria. These differential diagnoses should have been looked for in all the cases aforementioned. Since malaria test was done, the most likely hypothesis retained was a cross reaction with malaria when the test was positive. None of the patients seemed to have been vaccinated, otherwise we would have a profile with agglutinations to TH, AH and BH altogether.

Outside endemic regions, a single Widal test in a patient not vaccinated and not exposed to the disease may have a diagnostic value. In endemic zones however, the serological diagnosis is normally based on increasing titres of antigens O and H and the population has high levels of antibodies because of frequent exposure to various antigens (*Salmonella* sp., *Plasmodium* sp.) (Hamze et al., 1998; Parry et al., 1999; Olopoenia, 2000; Nsutebu et al., 2003; Massi et al., 2003; Rao et al., 1999; Tohme et al., 2002; Jumba et al., 1995; Khan et al., 2005). Hoffmann stated that the result of a first Widal test is virtually interpretable unless the sensitivity, specificity, and predictive values are specifically known for the laboratory performing the analysis (Abdullah et al., 2005). Duthie, and other authors had raised the necessity to have specific criteria for the interpretation of the serological profiles in endemic areas, given the high frequency of cross reactions (Duthie and French, 1990; Hosoglu et al., 2006; Ismail, 2006; Pak-Leong et al., 1998; Clegg et al., 1994; Coovadia et al., 1986; Rodriguez and Duenas, 1993). Pang has suggested as criteria to assess positive agglutinins O, a titre at least equal to 1/160, what Hamze assumed to lower the sensitivity (Hamze et al., 1998; Tikky and Savithri, 1983). Finally, 1/200 is the titre of agglutinin O widely used as recommended by Parry for more reliable results (Parry et al., 1999).

Conclusion

In 200 patients aged 5 to 69, we performed the Widal test and detected only 3 cases of infection by *S. typhi*. We

report here the serological profiles of the patients and propose the interpretation of the 42 intermediate cases. The high rate of these intermediate serological profiles is scarring, since the Widal test appears to be the analysis the most requested in typhoid fever suspicion and no interpretation is provided by many laboratories. This case report could be useful to the laboratories as examples of interpretation. It strongly recommends a clinical examination prior to the serological test and interpretation regarding clinical outcomes and further procedures. Theses occurrences emphasize the need of a simpler and reliable immunologic test for the diagnosis of typhoid fever.

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