Interference by Malaria in the diagnosis of typhoid using Widal test alone

M. E. Ohanu[1], A. U. Mbah[2], P. O. Okonkwo[2] and F. S. Nwagbo[3]

[1]Department of Medical Microbiology, University of Nigeria Teaching Hospital, Enugu
[2]Department of Pharmacology, University of Nigeria, Enugu Campus
[3]Department of Community Medicine, University of Nigeria, Enugu Campus/

Summary

A total of 270 febrile patients (130 males and 140 females) aged between 15 and 59 were screened using thick and thin blood film stains for malaria, bacteriologic culture and Widal test for enteric fevers. Sixty (22%) were positive for malaria while 38 (14%) were positive for enteric fevers out of which 16(26.6%) concomitantly had malaria parasite. Cases without malaria parasite (MP) or enteric fever organism were 172 (63.7%) and classified as pyrexia of unknown origin (PUO). Forty-four were strictly malaria cases out of which 36 (82%) were due to *Plasmodium falciparum*, and all had antibody Widal titres ≥ 160 to 0 antigen while 4 (9%) were due to *Plasmodium malariae*, 3(6.8%) were due to *P. ovale* and 1(2.3%) was due to *P. vivax*. Twenty (52.6%) of the 38 patients with enteric fever had typhoid, all had Widal titres ≥ 160 to 0 antigen. In all, antibody reaction Widal titres to H antigen were <20. There was no statistical significant difference [X^2 = 327.2, P>0.05] between Widal titres of malaria and typhoid cases. Hence using Widal test alone, one cannot differentiate typhoid fever from malaria. In another 250 healthy adults, of equal sex distribution, used as controls 12(4.8%) had malaria parasite and 4(1.6%) had enteric fever organisms. While only 4(1.6%) gave Widal titre of 80 to 0 antigen the rest had antibody titres of <20 to O antigen. Malaria could interfere with serological diagnosis of typhoid and hence lead to over diagnosis of typhoid in Nigeria.

Keyword: ***Typhoid, Malaria, Widal test***

Résumé

Au total, 270 malades fébriles (130 hommes et 140 femmes) âgés entre 15 et 59 ont été passés par un test de dépistage tout en utilisant la tâche de sang épaisse et mince pour le paludisme, la culture bactériologique et le test de widal pour des fièvres typhoides. Soixante soit 22% étaient positifs pour le paludisme tandis que 38. soit 14% étaint positis pour des fièvres typhoides parmi lesquels 16 soit 26,6% avaient le parasite du paludisme concomitant.

Des cas sans le parasite du paludisme (AP) ou organisme de la fièvre typhoide étaient 172 soit 63,7% et classés comme pyrexie d'origine inconnue (POI). Quarante-quatre sont des cas puremet du paludisme parmi lesquels 36 soit 82% etaient causé par P. vivax. Vingt soit 52,6% des 38 patients atteints des fièvres typhoides avaient la typhoide, tous avaient titres Widal >/ 160 à 0 antigène.

Dans l'ensemble, l'effet d'anticorps titres widal par rapport à H antigène étaient <20.

II n'ya aucune différence statistiquement importante (x2 = 327,2, P>0, 05) entre titres Widal de paludisme et cas typhoides. Donc, à travers l'utilization du test widal seulement, on ne peut pas différencier les fièvres typhoides de paludisme. Chez des autres 250 adultes bien portants d'une distribution égale en matière du sexe, utilisé comme contrôles 12 soit 4,8% avaient le parasite du paludisme et 4 soit 1,6% avaient des organisms de fièvres typhoides. Tandis que 4 soit 1,6% seulement avaient donné titre widal de 80 à 0 antigène, les autres avaient titres d'anticorps de <20 à 0 antigène. Le paludisme pourrait interférer avec le diagnostic sérologique de la typhoide et ainsi abouti au diagnostic excessif de la typhoide au Nigeria.

Introduction

Enteric fevers comprise of typhoid and paratyphoid fevers, which require prompt antibiotic treatment.[1] In Nigeria most of the typhoid cases are diagnosed on the basis of clinical symptoms and a single Widal test performed in hospitals and private laboratories.[2] Blood culture is seldom done. Hence the reliability of diagnosis based on Widal test along is often questionable. There are symptoms of other infections, which mimic those of enteric fever. These include brucellosis, malaria, and hepatitis.[3] Diagnosis of enteric fever infections based solely on Widal test could be unreliable.[4] Also malaria and typhoid can co-exist in the same patient creating problems in diagnosis.[5]

There is a paucity of reports on microbial investigations on enteric fevers in Nigeria and there is hardly any report on simultaneous screening of patients for malaria and enteric fever with bacteriological proof.

Material and Methods

Enugu is in the savannah region of Nigeria and is surrounded by hills mainly at the northern and western borders. Patients with pyrexia attending University of Nigeria Teaching Hospital Enugu clinics and suspected of having malaria or enteric fever were included in this study. The study was carried out between November 1997 to October 1998. The inclusion criteria were fever, abdominal pain or discomfort, constipation, diarrhoea or tarry stool of not less than two days. Mercury thermometers were used to check that patients' temperatures were above 37°C unless they had taken some analgesic before coming to the clinic. Patients on antibiotics were excluded from the study. Blood, urine and stools samples were collected from all the patients before any therapy was instituted.

Eighteen ml. of blood was drawn from each individual following standard procedure for blood culture. Five ml. each for aerobic and anaerobic culture, 3ml was put into a tube containing EDTA and used for blood films. Another 5ml was allowed to clot and the serum used for Widal test.

Blood cultures were performed in brain heart infusion and thioglycolate media and subcultures done on blood agar.

Stool samples were collected in sterile wide mouthed universal containers and urine samples in sterile universal containers with boric acid. They were first enriched in selenite F-broth before inoculation onto MacConkey and Deoxycholate agars by wire loop streaking method.

The resulting growths from all cultures were examined for *Salmonellae* as described by Hawkey and Lewis.[6]

Thick and thin blood films of all the patients were stained using Field's stain to check for malaria parasite.[7] Also Widal agglutination test using febrile antigens of *Salmonella* (made by Gamma biological Houston USA) were done on sera of all the patients using tube dilution method described by Freter.[8]

Additional 250 normal healthy adults as controls were subjected to Widal test, stool culture for *Salmonellae* and their blood films also checked for malaria parasites.

Results

From November 1997 to October 1998, a total of 270 patients (130 males and 140 females), 250 health controls of equal sex distribution were involved in the study. All patients and controls were screened for typhoid and malaria. Only 38(14.1%) patients were positive for typhoid and paratyphoid fevers, mainly through blood and stool cultures out of which typhoid cases were 20(52.6%) with Widal titres to 0 antigen ≥ 160. (Table 1). Only two urine

* *Correspondence*

samples were culture positive for typhoid.

Table 1 Enteric and Malaria fever prevalence

Cases	No.	Percentage
Enteric fever	38	14
Malaria	60	22
PUO	172	64
Total	270	100

Results of the blood films showed that 60(22.2%) were positive for malaria parasite and 16(26.6%) of these were bacteriologically proved to be coharbouring enteric fever organisms. All the 16 patients had Widal titres to 0' antigen $\geq$ 160 (Table 2). Of the malaria cases, 36 were caused due to *Plasmodium falciparum* and all had Widal titres of 160 and above to 0' antigen, while 4 were due to *P. malariae* 3, by *P. ovale*, and 1 by *P. vivax* with titres $\leq$ 160 (Table 3). There was no statistical significant difference ($X^2 = 327.2$, $P > 0.05$) between Widal titres of malaria and typhod cases.

Table 2 Widal reaction by cases of typhoid, malaria and enteric with malaria

Cases	No	Widal titres to 0 antigen					
		20	40	80	160	320	640
Typhoid	20	0	0	0	8	8	4
Enteric with malaria	16	0	0	0	3	10	3
Malaria only	44	0	0	7	25	12	0

Most of those with malaria parasite who had treatment for it did not have rising titre on repeat Widal and 50% had become non reactive within two weeks following treatment while those of typhoid were still reactive.

Table 3 Widal reaction by malaria cases according to species of parasite

Species	No of cases	Widal titres to 0 antigen					
		20	40	80	160	320	640
P. falciparum	36	0	0	0	26	10	0
P. malariae	4	0	0	2	2	0	0
P. ovale	3	0	0	1	2	0	0
P. vivax	1	0	0	1	0	0	0

Table 4 Widal reaction by cases of PUO and healthy controls

Cases	Total no.	Widal titres to 0 antigen					
		20	40	80	160	320	640
PUO	172	130	0	32	10	0	0
Controls	250	246	0	4	0	0	0

The remaining 172 patients without plasmodia and not culture positive for typhoid and paratyphoid organisms were classified as pyrexia of unknown origin (PUO) with regard to the topic of study. Their Widal titres did not exceed 80 (Table 4). In the 250 healthy controls of equal sex distribution 4(1.6%) had enteric fever organisms and gave titres up to 80 and these were to 0 antigen (Table 4). *Salmonella typhi* was isolated from two healthy controls and *S. paratyphi C.* was recovered from another one. Malaria parasites were seen in the blood films of 12(4.8%) of the controls.

Discussion

While a single Widal test could be used judiciously in the diagnosis of typhoid fever, it is not full proof hence bacteriological evidence is invaluable. Widal test is abused in Nigeria[9,2] and could be responsible for over diagnosis of typhoid fever[10] hence causing an epidemic scare. Symptoms of malaria can mimic those of typhoid. Again malaria can interfere in the diagnosis of typhoid especially if a single Widal test is used. This can create problems for the clinician. Most people can afford to pay for anti malaria drugs, but drugs for enteric fevers are either expensive or toxic e.g. Chloramphenicol to children and the elderly. Most malaria parasite positive cases responded well to good antimalaria therapy even when they had high Widal titres. Usually with a single Widal test in the private clinics, these cases are pumped with antibiotics without bacteriologic confirmation for enteric fever organisms.[11] The consequences of this abuse of antibiotics on the community could be far reaching.

It is probable that the quality of febrile antigens and technique affect the result of Widal tests, thus care should be taken to rule out malaria and other illnesses that could interfere. Again the phenomenon of anamnestic response further complicates the problem as antibodies unrelated to the causative organism could be produced in a febrile condition. Also an asymptomatic human carrier state-exists for the agents of typhoidal and non-typhoidal Salmonellosis. It is difficult to isolate typhoid organisms from carriers, as many attempts have to be made at culture. Hence a carrier suffering from malaria could have raised antibody titre to *Salmonella* antigens enhanced by anamnestic response. Carriers may thus contribute to those giving Widal reaction in PUO group. The cases of *P. falciparum* were in acute febrile condition and their Widal titres when repeated did not show any increase. Their antibody titres to 0 antigen after two weeks fell to 20 instead of the expected increase as seen in most typhoid cases. In addition to good clinical judgement, it is wise to repeat suspect Widal reactions two weeks later. We suggest that febrile patients with a high single Widal titre, should be treated first with first line antimalaria drug. In resistant cases, second line antimalaria drug should be used. Having excluded malaria, treatment consideration against enteric fevers can then be given.

Conclusion

The gold standard for the diagnosis of typhoid or paratyphoid fevers is bacteriological proof. If Widal test must be used, a repeat test must be done after a week to check for rising titre. When only a single Widal test is used, malaria can interfere in the diagnosis of typhoid.

Acknowledgements

The author is grateful to J. Ofunne, N. Ibecheozor, C. Nlemadim and R. Osefo, for their assistance in the laboratory work. E. N. Shu reviewed the manuscript.

References

1. Giannella R A: In Medical Microbiology Baron S. Editor 1991 3rd ed. Churchill Livingstone, New York 317 - 325.
2. Onuigbo M A C: Typhoid fever epidemic in Nigeria? The abuse of the Widal test and the antibiotic Chloramphenicol. Nig Med. Pract 19989; 18: 23 - 25.
3. Khan W, Maldonado S, Akram S, Gardner M and Rodriquez W: Rapid screening for Salmonella directly from stools by Dipstick Enzyme-linked Immunosorbent assay. ASM Annual Meeting, Dallas tex 5 - 9 May, 1991.
4. Onuigbo M A C: diagnosis of typhoid fever in Nigeria: misuse of the Widal test. Trans, Roy Soc. Trop Med & Hyg. 1990; 84: 129 - 131.
5. Ammah D, Nkuo T, Ndip R and Deas J E: An update on concurrent malaria and typhoid fever in Cameroon. Trans Roy. Soc Trop. Med & Hyg. 1999; 93: 127 - 129.
6. Hawkey P M and Lewis D A: Medical bacteriology, a practical approach 1989 1st Ed. Oxford University Press: 21 - 42.
7. Field J W: Further notes on the method of staining malaria parasites in thick blood films. Roy. Soc. Trop. Med Hyg. 1941-42; 35: 35.
8. Freter R: Agglutination titration (Widal) for the diagnosis of enteric

fever and other enterobacterial infections. In Manual of Clinical Immunology 1976, Rose N and Friedman H (editors). Washington D. C. American society for microbiology.

9. Animashaun T and Odugbemi T: Widal test: Use and abuse in Nigeria. Nig. Med. Pract 1990; 19: 96 - 98.

10. Mohammed I, Chikwem J O and Gashau W: Determiantion by Widal agglutination of the base line titre for the diagnosis of typhoid fever in two Nigerian State. Scand. J. Immunol 1992; 36: 153 - 156.

11. Osuafor T O K, Anyiwo C E: A review of Widal test results at Nnamdi Azikiwe University Teaching Hospital, Nnewi Nig. Med. J. 1995; 28: 39 - 40.