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Determination of baseline antibody titre to *S. typhi/paratyphi* in Ile-Ife, Nigeria

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Summary

The aim of the study is to determine the baseline antibody titres to *Salmonella typhi / paratyphi* in healthy individuals with a view to establishing the significant titre for Widal agglutination test in Ile-Ife, Western Nigeria. Three hundred and ten healthy volunteers were admitted into the study and forty-eight clinically diagnosed and culture positive cases of Enteric fever were used as purposeful controls. Widal test was performed on each serum. Slide agglutination test was first done, then positive samples were further subjected to tube agglutination for quantitative titration. Of the 310 healthy volunteers, 274 (97.2%) had reciprocal antibody titre of $\leq$80 to 0 antigen and 265 (95.0%) had reciprocal antibody titre of $\leq$ 80 to H. antigen of *S. typhi*. On the other hand, in 48 control cases, 43 (89.6%) had reciprocal antibody titre of $\geq$ 160 to O antigen and 38 (78.2%) had reciprocal titre of $\geq$160 to H. antigen. The sensitivity and specificity of the test were 89.9% and 94.2% for O antibody, and 79.2% and 92.3% for H. antibody respectively. The baseline titre to *S. typhi / paratyphi* for both O & H antibody is 1:80 whilst the significant titre for O & H antibodies is 1:160 and above in Ile-Ife, Nigeria.

Keywords: *Baseline titres, enteric fever, Ile-Ife.*

Résumé

Le but de cette étude est de determiner la base d'anticorps titres au salmonella typhi/ paratyphi sur les individus normaux ayant pour but d'établir la significance titre de l'examen de l'agglutination Widal a Ilé –Ifé, ouest du Nigeria. Trois cent trente volontaires étaient admis dans cette étude et quarante huit étaient diagnostiqués cliniquement et leur culture positive de la fiévre enterique étaient utilisées comme contrôle.Les testes widal etaient faite sur chaque echantillon de serum. La lame du teste d'agglutination etait faite premierement, apres les echantillons positives etaient soumisse au tube d'agglutination pour titration de quantification. Sur les 310 volontaires, 274(97.2%) avait le titre de anticorps reciproque de < 80 de H antigene a 0 antigene et 265 (95.0%) avait le titre d,anticorps reciproque de <80 a l'antigene H. du S typhi. De l'autre cote, sur 48 cas de contrôle, 43 (89%) avait le titre d anticorps reciproque > a 160 a l antigene 0 et 38(78.2%) de titre reciproque > 160 a l'antigene H. La sensitivite et la specificite de cet examen 89.9 % et 94.2% pour l anticorps 0 et 79.2% et 92.3% pour l'anticorps H. respectivement. La base de titratition du S typhi et paratyphi pour les anticorps 0 et H est 1 : 80 alorsque la sificance du titre pour les anticorps 0 et H est plus de 1 :160 dans la Ville d'Ile-Ife, Nigeria

Introduction

Typhoid fever remains an important health threat in many parts of the world, with an estimated 16 million cases and 600,000 deaths occurring each year [1].

There are many reports from African countries including Nigeria of misdiagnosed typhoid fever cases based on a single pre-treatment Widal agglutination test [2,3]. There are often great problems in interpreting the Widal test results in patients who have previously had typhoid immunization and in those who live in typhoid endemic areas where raised antibody titres are commonly found, which do not represent current enteric fever [4]. The minimum (baseline) agglutination titre must therefore be determined in individual geographical areas. The Widal test result is most reliable in areas where baseline titre in a control groups of healthy individuals in all geographical areas are available [5-9].

When the baseline titre to *Salmonella typhi and paratyphi* in a particular geographical location is determined, this will aid in establishing the significant Widal test titre (diagnostic titre) which subsequently, will help the clinicians to interpret Widal test result correctly. Some investigators have already established diagnostic Widal test titres in some countries [2,13] and are being used in the diagnosis of enteric fever in patients that have clinical signs and symptoms compatible with enteric fever.

Definitive diagnosis of enteric fever depends on isolating the aetiological agents from clinical specimens. When it is not possible to isolate Salmonella from specimens, presumptive diagnosis can be provided by the serological method (Widal reaction) and chemical method (diazo reaction) [10].

The importance of early diagnosis, prompt and effective treatment of enteric fevers can not be overemphasised. These can prevent the spread of the disease and subsequent carrier state. More importantly, it will reduce the mortality and some complications of the disease [4].

The main aim of the study is to determine the baseline antibody titres to *S. typhi / paratyphi* in apparently healthy individuals and selected clinically diagnosed or laboratory proven enteric fever cases with a view to establishing the significant titres of Widal test in Ile – Ife Nigeria.

Materials and methods

The study was conducted at the Department of Medical Microbiology and Parasitology of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria. The town is located in the South-Western part of the country. The samples were obtained from healthy individuals at Obafemi Awolowo University (OAU), OAUTHC, and healthy volunteers in the town in order to cover many socio-economic groups. It is a descriptive study, and stratified random sampling method was employed to select participants in different target populations. Subsequently, simple random sampling was applied to each stratum. Sample size was determined using the Computer Programmes for Epidemiological Analysis (CPEA) of 1995. Apparently healthy Volunteers of 12 years and above were recruited into the study. Exclusion criteria include previous history of typhoid immunization, febrile illness with abdominal

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pains, diarrhoea or constipations within the last 6 months and chronic liver disease.

With the aid of questionnaire, relevant information was obtained from both the subjects and the patients.

Five mililitres of venous blood was collected from each subject and the serum was used for widal agglutination reaction. The test was performed first using slide agglutination method, then the positive samples were subjected to quantitative standard tube dilution method. The antibody titres to somatic 'O' and flagellar 'H' antigen were determined using Febrile Antigen Kits, manufactured by Antec Diagnostic Product United Kingdom. Antibody against Vi antigen was not tested for.

The Data were analysed using statistical package for social sciences(SPSS) software and the sensitivity, specificity, positive and negative predictive values and test efficiency were calculated.

Results

The results of antibody agglutination titres against various antigens of *S. typhi* and *S. paratyphi* in 310 healthy subjects and 48 enteric fever patients are shown in tables 1 and 2 respectively.

Table 1: O and H antibody titres of 310 healthy subjects

Organism	Type of Ag.	Reciprocal antibody titres (% in parenthesis) <20	20	40	80	160	320	640	Total
S. typhi	O	243 (78.4)	15 (4.8)	8 (2.5)	8 (2.5)	8 (2.5)	0	0	282 (90.9)
S. paratyphi A	O	0	0	0	0	1 (0.3)	0	0	1 (0.3)
S. paratyphi B	O	0	2 (0.6)	2 (0.6)	12 (3.9)	7 (2.3)	2 (0.6)	0	25 (8.1)
S. typhi + S. paratyphi B	O	0	1 (0.3	1 (0.3)	0	0	0	0	2 (0.6)
S. typhi	H	243 (78.4)	18 (5.8)	11 (3.5)	20 (6.5)	16 (5.2)	2 (0.6)	0	310
S. paratyphi A	H	197 (63.5)	17 (5.5)	16 (5.2)	35 (11.3)	8 (2.5)	4 (1.3)	2 (0.6)	279 (90)
S. paratyphi B	H	0	1 (0.3)	1 (0.3)	0	1 (0.3)	0	0	3 (0.9)
S. paratyphi A + B	H	0	1 (0.3)	3 (0.9)	5 (1.6)	2 (0.6)	0	0	11 (3.5)
S. paratyphi C	H	0	0	0	2 (0.6)	4 (1.3)	1 (0,3)	0	7 (2.3)
S. paratyphi A + S. paratyphi B	H	0	0	0	1 (0.3)	2 (0.6)	0	0	3 (0.9)
S. paratyphi A + C	H	0	0	0	0	1 (0.3)	0	0	1 (0.3)
S. typhi + paratyphi B	H	0	0	0	0	0	4	1	5(1.6)
Total		197 (63.5)	20 (6.5)	23 (7.4)	46 (14.8)	17 (5.5)	5 (1.6)	2 (0.6)	310

The data analysis shows that in 310 healthy subjects studied, 274 (97.2%)had reciprocal antibody titres of ≤ 80 to *S. typhi* O antigen and 265 (95.0%) had reciprocal antibody titres of ≤ 80 to H antigen. Only 8 (2.8%) of the healthy subjects had antibody titres of 1:160 to *S. typhi* O antigen and 14 (5.0%) had titres of ≥ 1:160 for H antibody (table I). *S. paratyphi* also shows similar trend.

Table 2: O and H antibody titres of S. typhi/paratyphi of 48 enteric fever patients (control).

Organism	Type of Ag.	Reciprocal antibody titres (% in parenthesis) 20	40	80	160	320	640	1280	Total
S. typhi	O	1 (2.1)	1 (2.1)	2 (4.2)	9 (18.8)	10 (20.8)	12 (25)	5 (10.4)	40 (83.3)
S. paratyphi A	O	0	0	0	1 (2.1)	0	1 (2.1)	0	2 (4.2)
S. paratyphi B.	O	0	0	1 (2.1)	2 (4.2)	2 (4.2)	1 (2.1)	0	6 (12.5)
Total		1 (2.1)	1 (2.1)	3 (6.5)	12 (25)	12 (25)	14 (29)	5 (10.4)	48
S. typhi	H	2 (4.2)	4 (8.3)	4 (8.3)	11 (22.9)	10 (20.8)	8 (16.7)	1 (2.1)	40 (83.3)
S. paratyphi A	H	0	0	0	2 (4.2)	0	0	0	2 (4.2)
S. paratyphi B	H	0	0	0	4 (8.3)	2 (4.2)	0	0	6 (12.5)
Total		2 (4.2)	4 (8.3)	4 (8.3)	17 (35.4)	12 (25)	8 (16.7)	1 (2.1)	48

Of the 48 enteric fever patients, 43(89.4%) had reciprocal antibody titre of ≥ 160 against O antigen and 38(79.2%) had reciprocal antibody titres of ≥ 160 to H antigen. *S. paratyphi* shows similar pattern. It has been shown that, the pattern of agglutination reaction in 310 healthy subjects is waning at reciprocal titre of 160, whilst in 48 enteric fever patients, there is a sharp peak at a reciprocal titre of 160 in both O and H antibodies (tables 1 and 2).

Table 3: O and H agglutination titres of 21 culture proven typhoid fever patients.

Type of antigen	Reciprocal antibody titres (% in parenthesis) 20	40	80	160	320	640	1280
O	0	0	1 (4.8)	8 (38.1)	5 (23.8)	2 (9.5)	5 (23.8)
H	0	0	3 (14.3)	6 (28.6)	8 (38.1)	4 (19.0)	0

Of the 48 clinically diagnosed cases of enteric fever patients, 21 had culture positive to salmonellae from blood and stool specimens (table 3). The table shows 20 (95.2%) typhoid fever patients had reciprocal O antibody titres of 160 and above, and only 1 (4.8%) had reciprocal antibody titre of 80 and non

has less than 80. For the H antibody titres 18 (85.7%) typhoid fever patients had reciprocal titres of 160 and above and only 3 (14.3%) had reciprocal antibody titres of 80, non has less than 80.

Table 4: Significant O antibody titre of >1:160

	Widal test Positive	Widal test negative	Total
No. patients with disease	43 (TP)	5 (FN)	48
No. of subjects without disease	18 (FP)	292 (TN)	310
Total	61	297	358 GT

Key
TP = True positive
TN = True nagative
FP = False positive
FN = False negative
GT = Grand total

The sensitivity and specificity of the test were 89.9% and 94.2% for O antibody, while for H antibody, the sensitivity and specificity were 79.2% and 92.3%. The positive and negative predictive values were 70.5% and 98.3% for O antibody, while for H antibody were 61.3% and 96.6% respectively. The test efficiencies were also calculated and found to be 94.9% and 90.5% for O and H antibodies(table 4).

Table 5: Significant H antibody titre of >1:160

	Widal test Positive	Widal test negative	Total
No. patients with disease	38 (TP)	10 (FN)	48
No. of subjects without disease	24 (FP)	286 (TN)	310
Total	62	296	358 GT

Key
TP = True positive
TN = True nagative
FP = False positive
FN = False negative
GT = Grand total

Table 6: Diagnostic accuracy of using significant antibody titres of >1:160 in both O and H antibodies.

Widal test antigen dilution	Sensiti ty (%)	Specifi city (%)	Positive predictive value (%)	Negative predictive value (%)	Test efficiency (%)
O≥1:160	89.9	94.2	70.5	98.3	94.9
H≥1:160	79.2	92.3	61.3	96.6	90.5

Discussion and conclusion

Of the 310 healthy subjects, 274(97.2%) had reciprocal antibody titre of ≤80 to O antigen for S typhi and 265 (95.0%) had reciprocal antibody titre of ≤80 to H antigen (table 1). This shows that majority of the subjects had reciprocal antibody titre of ≤80 to *Salmonella typhi*. On the other hand, table 2 shows that among 48 clinically diagnosed enteric fever patients 43 (89.6%) had reciprocal antibody titres of ≥ 160 to O antigen and 38(79.2%)had reciprocal antibody titres of ≥ 160 to H antigen . The table shows a sharp increase in the number of patients at the serum dilution of 1/160. This study shows that the baseline antibody titre to *S.typhi/paratyphi* in Ile-Ife is 1/80 for both O and H antibodies. The baseline antibody titre obtained in our study is similar to that of Maiduguri and Jos [12]. This is probably because our study geographical area and population lie in the same latitude and are similar in both social and health set up.

Table 3 shows a breakdown of titres for 21 culture proven cases of typhoid fever among the 48 diagnosed clinically. 20(95.2%) and 18 (85.7%) had reciprocal O and H antibody titres of ≥ 160 respectively. Only 1(4.8%) patient and 3(14.3%) had antibody titres of 1:80 against O and H antigens respectively. Considering the results obtained in both subjects and patients groups the titre tapered off at 1:160 in the healthy group while there is a peaking up of number positive in the patients group at 1:160. In both the subjects and control groups the meeting point is 1:160. Therefore the significant (diagnostic) Widal 0 antibody titre can be considered as from 1:160 and above.

With a sensitivity of 89.9% and 79.2% and specificity of 94.2% and 92.3% for anti O and H antibodies respectively (table 6), one can safely say that the significant (diagnostic) titres for enteric fever in Ile-Ife using Widal agglutination reaction is 1/160 for both O and H antibodies in a patient whose clinical presentation is compatible with enteric fever, the anti O titre being more important diagnostically than anti H titre. This finding is in keeping with the two studies done in Maiduguri and Jos, Nigeria and that of Kumasi, Ghana [2,12].

Single pre-treatment serum can be used to diagnose *typhoid* fever in patients with clinical signs and symptoms suggestive of enteric fever if the baseline titre is determined and significant titre is established [2,12,13,14]. In this study, 41(84.4%) of the 48 enteric fever patients had fever for at least a week duration, therefore presence of fever for at least one week duration is an added support if single pre-treatment serum is to be used in the diagnosis of enteric fever, if the O antibody titre is ≥160, and other causes of fever are eliminated.

When compared with findings outside the country, our findings are similar to those from Ethiopia, Egypt and Kumasi Ghana. Nevertheless our significant antibody titre of ≥1/160 is less than the finding in Sudan, South Africa and Malaysia where the significant titres are ≥1/320, ≥1/200 and ≥1/320 for both O and H antibodies [2]. The significant titre in Singapore is ≥1/40 a figure much more lower than the finding in this study [2]. The differences in significant antibody titres in different geographical locations probably depend on the endemicity of the infection in the areas which also depends on the sanitary condition of the area, availability of adequate and safe drinking water and level of food hygiene [15].

In the healthy population group more subjects 14(4,4%) have H antibody titres of ≥160 to *S typhi* than those8(2,5%) with O antibody titres of≥160 (table I). This is probably due to the longer persistent of H antibody in the circulation after infection. For *S paratyphi* equal number of subjects 10(3.2%) have reciprocal O and H antibody titres of ≥160. This could probably be due to sub clinical infection or cross reaction

by nontyphoidal Salmonellae or other coliforms [16] (table 3).

Among 310 healthy subjects, 8(2.5%) have reciprocal O antibody titre of 160 and 14(4.4%) have reciprocal H antibody titre of ≥160 against *S. typhi*. Considering the sensitivity and specificity, positive and negative predictive values and efficiency of the test these could be considered false positive. These false positive results could probably be due to cross-reaction by nontyphoidal salmonellae and other coliforms, or due to sub clinical infection because of the endemicity of *S. typhi* infection in the geographical location of the study centre [16]. Other causes of false positive Widal agglutination reaction include; Malaria [3], Brucellosis [17], typhoid immunization and hyperglobulinaemic conditions [18].

On the other hand, among 48 typhoid fever patients, only 4(8.3%) had reciprocal O antibody titre of ≤80 and 10(20.8) have reciprocal H antibody titre of ≤80 (table 2). These are false negative results, which could probably be due to early treatment with chloramphenicol, which may halt a rise in the antibody levels [13], or any appropriate antibiotic intervention early in the course of the infection, which may inhibit further production of antibodies [16]. The chances of an error in diagnosis could never the less be totally ruled out.

In conclusion, the baseline antibody titre to *Salmonella typhi/paratyphi* in Ile-Ife has been determined as 1/80 for both O and H antibodies. For a single pre-treatment Widal agglutination reaction to be significant or diagnostic in clinically suspected cases of enteric fever in Ile-Ife, the antibody titre against O and H antigens or O antigen alone should be ≥1/160.

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