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Evaluation of the Performance of Widal Slide Agglutination Test Compared to Blood Culture and Evaluation of Interferon Gamma Response in the Diagnosis of Typhoid Fever

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Abstract

Typhoid fever remains a public health challenge in developing countries including Nigeria. Widal test is widely used for the diagnosis of typhoid fever due to its simplicity and short turnaround time. However, the specificity of this test has been debated. The aim of the study was to evaluate the performance of Widal test compared to blood culture and determine interferon gamma response among the study subjects. Blood samples were collected from 90 patients who complained of fever and other symptoms suggestive of typhoid fever. Widal slide agglutination test, automated blood culture and interferon gamma concentrations were conducted using rapid antibody detection kit, BACTEC and sandwich enzyme linked immunosorbent assay (ELISA) respectively. Of the 90 samples tested, 63 (70.0%) were positive for anti-Typhi O antigen while 42 (46.7%) were positive for anti-Typhi H antigen. Similarly, 18 (20%) of the blood samples were non- S. Typhi culture positive while 72 (80%) had no bacteria isolated. None of the cases had S. Typhi positive culture. With regards to interferon gamma, subjects with lower levels of 15pg/mL had no bacteria isolated from their blood. As the interferon gamma concentration increased, more subjects had non- S. Typhi bacteria isolated from their blood which shows the relationship between interferon gamma and bacteraemia. The study demonstrated that the use of Widal serology test in the diagnosis of typhoid fever may be erroneous as all the samples were found to be negative for S. Typhi using the gold standard culture methods while Interferon gamma concentration was statistically related to the isolation of non- S. Typhi in blood culture as such, could be a good marker for the development of an alternative screening test, possibly an interferon gamma based detection system for typhoid fever. However, further research is recommended to elucidate that.

Keywords: *Typhoid, Widal test, Blood culture, Interferon gamma*

INTRODUCTION

Salmonella Typhi is a facultative, intracellular pathogen, which is widespread and poses a serious health challenge in developing countries (Fidan *et al.*, 2008). It is a well-known fact that typhoid fever has become endemic in developing countries including Nigeria causing a significant morbidity and mortality (Ajibola *et al.*, 2018; Chu *et al.*, 2011). World Health Organization (2018) estimates the global typhoid fever disease burden at 11-20 million cases annually, resulting in about 128,000-161,000 deaths per year. In a recent study by Abdulkarim and Mohammed (2017), about 1,225 cases of typhoid fever were recorded within five years in a single hospital in Kano State. Nas *et al*. (2017) also reported an 18% prevalence of typhoid fever in Kumbotso local government area of Kano state.

Salmonella serovars Typhi and Paratyphi are restricted to human hosts and cause systemic disease that can often be fatal (Pham and McSorley, 2015). A serious complication of typhoid fever is perforation of the intestine and remains a significant surgical problem in developing countries where patients usually perforated within 14 days of illness (Chalya *et al.*, 2012). The high incidence of perforation in most developing countries has been attributed to late diagnosis.

The diagnosis of typhoid fever is commonly made using Widal test which has since been proven to be neither sensitive nor specific (Ajibola *et al.*, 2018) due to cross reactivity of antibodies with similar antigens. T-cells on the other hand have single antigen specificity and hence, are not prone to cross-reactivity unlike antibodies. When primed with antigens, T- cells produce INF-γ in response to the antigen therefore, detection and quantification of interferon gamma (INF-γ) levels in these suspected subclinical cases may help establish

threshold levels of the cytokine that may be As previously documented, INF-γ has been used in the diagnosis of latent TB infection and found to be about 95% specific (Siegel *et al.*, 2018).

Early and definitive diagnosis of typhoid is therefore critical in avoiding fatal complications such as perforation of the intestines (Abbas *et al.*, 2014). The aim of study was to evaluate the performance of Widal test compared to standard blood culture and determine Interferon gamma response among the patients investigated for typhoid fever.

MATERIALS AND METHODS

The study was conducted at Aminu Kano teaching hospital (AKTH) and Murtala Muhammad specialist hospital (MMSH), Kano – Nigeria. Ethical approval was sought and obtained from the ethical committee, Aminu Kano teaching Hospital and Kano State Ministry of Health with reference numbers AKTH/MAC/SUB/12A/P-3/VI/2749 and MOH/Off/797/T.I/1424 respectively. The study population comprised of patients with clinical signs and symptoms suggestive of typhoid fever with request of Widal test from Physicians at MMSH.

A sample size of 90 was used in the study and was determined using the formula developed by Gberikon *et al*. (2019), based on previous prevalence of 4.5% of *Salmonella* Typhi infection in Kano State (4.5%) as reported by Akinyemi *et al*. (2018). A structured questionnaire was administered to obtain demographic characteristics and factors associated with S. Typhi infection among the study population.

Sample Collection and Processing

Using a sterile syring, exactly 10 ml of blood from each consented patient was aseptically collected; 7ml dispensed into the BACTEC aerobic plus culture vial and 3ml into a plain container which were both transported to the laboratory for further analysis (Andualem *et al.*, 2014).

Widal Serology

Widal slide agglutination test was done using S. Typhi, S. *paratyphi* A, S. *paratyphi* B and S. *paratyphi* C, O and H antigens according to the instructions of the manufacturer. The antigen suspension commercially available in 5 ml volume from Chronolab systems, (Barcelona, Spain) was used. A direct qualitative slide agglutination technique was used in this study for determination of the agglutination ability of sera (Deksissa and Gebremedhin, 2019).

Inoculation of Blood Samples

Blood samples collected were cultured using the BACTEC fluorescent series 9120 instruments (Becton Dickinson, USA) automated microbiology systems following the method described by Anvarinejad *et al*. (2016) with slight modifications. Exactly 7ml of blood was aseptically dispensed into the BD Bactec Plus aerobic/F culture vial containing soybean-casein digest broth and then loaded into the BACTEC machine within 30 minutes of sample collection. Whenever the machine gives an alert, the specific bottle was removed, Gram stained, and sub cultured on chocolate agar and MacConkey's agar. The isolates were identified as *Salmonella* based on Gram staining, the oxidase test, the catalase test, motility, triple-sugar iron (TSI) fermentation, and colony morphology. Negative were removed after the system confirms them negative while positives not confirmed to be S. Typhi were recorded as non- S. Typhi.

Interferon Gamma Assay using Sandwich Enzyme Linked Immunosorbent Assay (ELISA)

Interferon Gamma Quantification was done using the INF-gamma human ELISA kit from Melsin Medical Co. Limited (Cat. No. : EKHU-0162), following the manufacturer's guidelines. Absorbance of each well was read on a spectrophotometer using 450 nm as the primary wavelength. The standard curve with standard concentration on the x-axis and absorbance on the y-axis was plotted for interferon gamma to best-fit straight line through the standard points.

Data Analysis

A Four parameter Logistic (4PL) regression was used to plot a standard curve fit from which interferon gamma concentration of samples was interpolated. Statistical association between levels of interferon gamma, anti- S. Typhi antibody and blood culture results among the study subjects was determined at 95% confidence interval using Fisher's exact test with the aid of statistical package for social sciences (SPSS) version 22 where a P value of ≤ 0.05 was considered significant.

RESULTS

Socio-demographic information of patients investigated for typhoid fever is shown in table 1. It is clear that females (32, 35.6%) report to the hospital with complains of fever than males (58, 64.4%). The mean age of the patients is 25 years ± 1.35 standard error of mean. Most of the patients were between the ages of 17-20 years. More than half of the patients were married (49, 54.4%) followed by single (40, 44.4%) with divorced category (1, 1%) recording the least frequency. Based on level of education, patients with at most secondary school qualification had the highest frequency (39, 43.3%) while those with non-formal education had the least frequency (8, 8.9%).

Table 1 : Socio-Demographic Information of Patients and Blood Culture Status.

Parameters	No. (%) Examined	Culture Negative (%)	Non- S. Typhi Culture Positive (%)
Gender			
Male	32 (35.6)	25(27.8)	7(7.8)
Female	58(64.4)	47(52.2)	11(12.2)
Total	**90(100)**	**72(80)**	**18(20)**
Age (years)			
< 21	38 (42.2)	31 (34.4)	7 (7.8)
21-30	28 (31.1)	23 (25.6)	5 (5.6)
31-40	12 (13.3)	10 (11.1)	2 (2.2)
41-50	9 (10)	5 (5.6)	4 (4.4)
51-60	2 (2.2)	2 (2.2)	0 (0)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Marital Status			
Single	40 (44.4)	30 (33.3)	10 (11.1)
Married	49 (54.4)	41 (4.6)	8 (8.9)
Divorced	1 (1.1)	1 (1.1)	0 (0)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Educational Level			
Non-Formal	8 (8.9)	6 (6.7)	2 (2.2)
Primary	20 (22.2)	18 (20)	2 (2.2)
Secondary	39 (43.3)	32 (35.6)	7 (7.8)
Tertiary	23 (25.6)	16 (17.8)	7 (7.8)
Total	90 (100)	72 (80)	18 (20)
Occupation			
Self-employed	15 (16.7)	15 (16.7)	0 (0)
Civil Servant	15 (16.7)	12 (13.3)	3 (3.3)
Unemployed	4 (4.4)	3 (3.3)	1 (1.1)
Student	35 (38.9)	25 (27.8)	10 (11.1)
Full-time Housewife	21 (23.3)	17 (18.9)	4 (4.4)
Total	**90 (100)**	**72 (80)**	**18 (20)**

The clinical presentation among patients investigated for typhoid fever is summarized in figure 1. It is clear from the figure that fever, headache and fatigue were the most common modes of presentation.

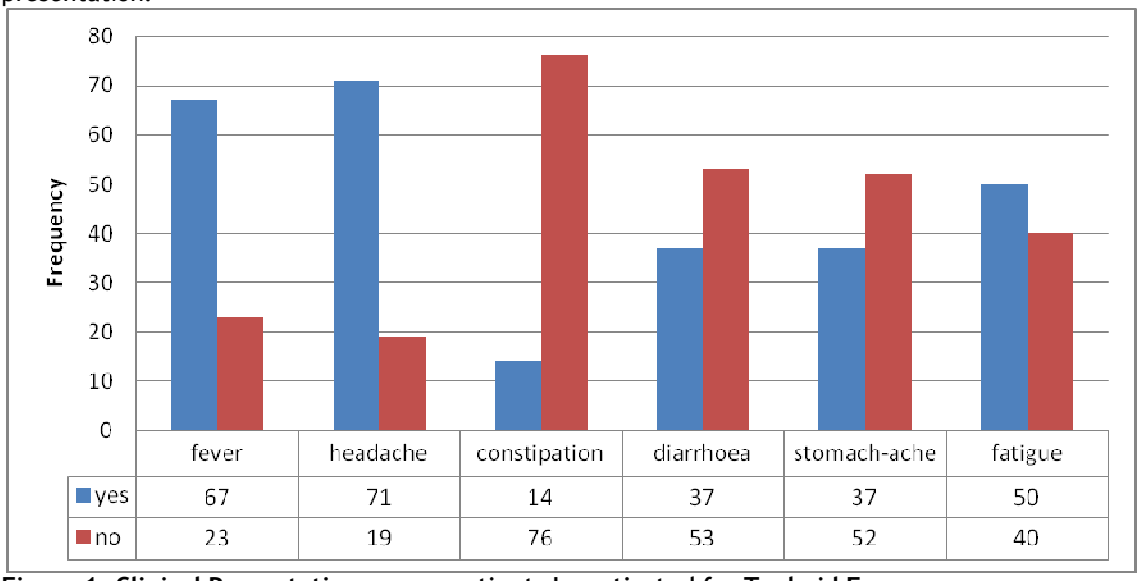

Figure 1: Clinical Presentation among patients Investigated for Typhoid Fever.

The results of the study revealed that out of the 90 blood samples tested, 63 (70.0%) were reactive to anti-Typhi O antigen while 42 (46.7%) were reactive to anti-Typhi H antigen (Table 2). Table 2 also show that, none of the cultured blood samples had *S.* Typhi, whereas 18 (20%) of them were positive for other non-*S.* Typhi bacteria.

Table 2: Prevalence of Typhoid Fever based on Widal and Blood culture Tests

Parameters	Number	Percentage (%)
Samples examined	**90**	**100**
Anti Typhi O		
Reactive	63	70
Non- Reactive	27	30
Anti-Typhi H		
Reactive	42	46.7
Non- Reactive	48	53.3
Culture status		
Positive S. Typhi	0	0
Negative S. Typhi	72	80
Positive Non- S. Typhi	18	20

The performance of Widal test compared to blood culture is shown in Table 3.It is clear that 42 (46.7%) were positive while 48 (53.3%) were negative for the O antigen, 63 (70%) of the patients tested were positive while 27 (30%) were negative for H antigen. Out of the 90 patients recruited, 72 (80%) had no positive blood culture while 18 (20%) had blood culture positive for non- salmonella bacteria.

Table 3: Performance of Widal Test Compared to Blood culture in the Diagnosis of Typhoid Fever

Widal-Test (Agglutination)		No. (%) Examined	Culture Negative (%)	Non-*S.* Typhi Culture Positive (%)	*P-Value*	OR
Anti-Typhi Reactive	O	63 (70.0)	52 (57.8)	11(12.2)	0.358	1.655
Anti-Typhi Non-Reactive	O	27 (30.0)	20 (22.2)	7 (7.8)		
Total		**90(100)**	**72 (80)**	**18(20)**		
Anti-Typhi Reactive	H	42 (46.7)	38 (42.2)	4 (4.4)	0.033	3.912
Anti-Typhi Non-Reactive	H	48 (53.3)	34 (37.8)	14 (15.6)		
Total		**90(100)**	**72 (80)**	**18(20)**		

Key: OR= Odds Ratio.

The relationship between Interferon gamma (INF-γ) concentration and blood culture status among the patients studied is presented in Figure 2. INF-γ concentration was found to be directly proportional to non – *S.* Typhi blood culture positivity among the studied population as subjects with lower interferon gamma levels recorded lower incidence of bacteraemia while those with higher IFN -γ recorded higher incidence of bacteraemia. Additionally, Interferon γ concentration was found to be between statistically associated with blood culture result (p=0.003).

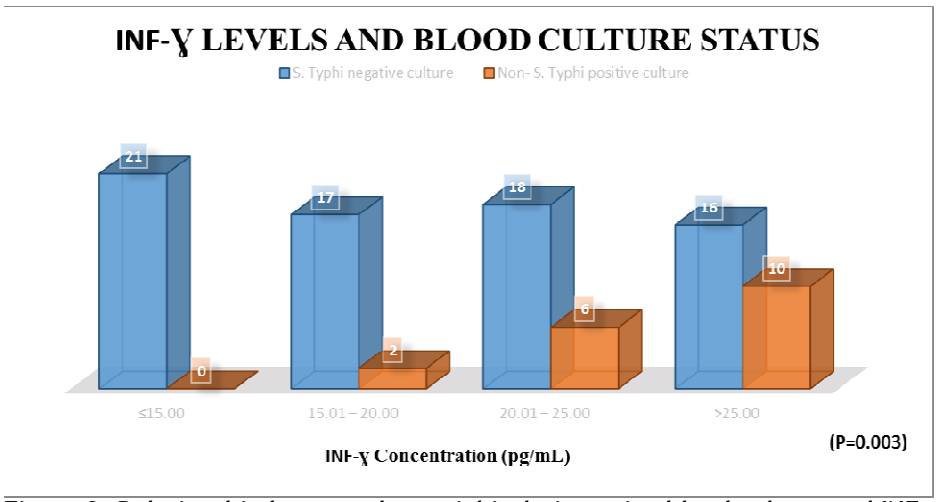

Figure 2: Relationship between bacterial isolation using blood culture and INF-γ level in the study population

DISCUSSION

Typhoid fever remains a public health problem in developing countries and its diagnosis on clinical ground is often difficult. Diagnosis in developing countries is typically done using Widal test. However, the value of the test has been of great concern.

From the present study, the prevalence of typhoid fever based on Widal slide agglutination test was found to be 70% and 46.7% based on reaction to *S.* Typhi O antigen, and *S.* Typhi H antigen. This is in agreement with the result of Deksissa and Gebremedhin (2019) who recorded a seroprevalence of 49.5% for both *S.* Typhi O and H antigens. However, no single *S.* Typhi isolate was recovered from patient's blood using blood culture which is the gold standard. The findings of this research are in agreement with that of Igiri *et al.* (2018) where they determined the prevalence of enteric fever in Ahmadu Bello university teaching hospital Zaria and found 70% prevalence using Widal but 0% using standard blood culture.

The observed disagreement between blood culture and Widal result reported in this study might be due to the fact that Widal test is based on the reaction of *S.* Typhi antigens (O and H) to specific antibodies in patient's serum where cross reactivity with similar antigens is possible. Cross reactivity of widal has been reported in patients with dengue fever by Bhatti *et al.* (2015) where they recruited patients who had clinical and serological evidence of dengue virus infection and subjected them to widal screening test. They found about 33% of the patients with dengue fever to be widal positive with no single *Salmonella* Typhi bacterium isolated from their blood. A similar trend was also recorded in this research where other bacteria were isolated from the blood of 18 (20%) of the patients tested. High seropositivity of widal might also be due to cross reactivity of the antigens to antibodies against malaria due to its endemic nature in Nigeria, leading to misdiagnosis and treatment of the wrong disease by physicians (Igiri *et al.*, 2018). This is also supported in this study as, revealed by the clinical history where most of the patients had no specific symptoms of typhoid fever like constipation, diarrhoea and stomach-ache.

Patients are usually diagnosed and treated for enteric fever wrongly by Widal test, of which its sensitivity has been debated. Blood culture which is a gold standard in typhoid fever diagnosis is not always available and, when available, it takes about 3 to 5 days. As a result, diagnosis may be delayed or overlooked and patients without enteric fever may receive unnecessary and inappropriate antimicrobial treatment due to the heavy dependence of rapid diagnosis using clinical features and serological methods (Deksissa and Gebremedhin, 2019). Therefore, rapid tests with better sensitivity and specificity are needed for the diagnosis of enteric fever.

This study demonstrated a strong correlation between serum interferon gamma levels and bacteraemia among the study participants (P=0.003). The study findings indicated that increased level of INF-γ concentration is associated with higher prevalence of non-*S.* Typhi bacterial isolates. This was evidenced by lower levels of ≤15pg/mL of INF-γ concentration reported against the negative blood samples compared to higher levels of >25pg/mL recorded by higher prevalent samples (Figure 2).

CONCLUSION

The study demonstrated that 70% and 46.7% of the studied blood samples were reactive to *S.* Typhi O antigen and H antigen using Widal slide agglutination test. However, none of the samples was positive for *S.* Typhi using blood culture, although 20% were identified as non-*S.* Typhi. The study further demonstrated that increased level of INF-γ concentration was significantly associated with higher prevalence of non-*S.* Typhi bacterial isolates. The study identified that the Widal serology test which is widely used in the diagnosis of typhoid fever may be erroneous as all the samples were found to be negative for *S.* Typhi using the gold standard culture methods. Based on the findings, the study recommend the development of an alternative screening test, possibly an interferon gamma based detection system for diagnosis of typhoid fever.

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