Comparison of Widal Test and Stool Culture Technique in Diagnosing Typhoid Fever in Okpokwu Local Government Area, Benue State, Nigeria

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ABSTRACT

Patients comprising of five hundred and sixty-nine (569) males and nine hundred and ten (910) females who attended primary health care units from the Northern Edumoga, Southern Edumoga (Ugbokolo) and Okpoga/Ichama areas were screened for Salmonella typhi and Salmonella paratyphi over a period of nine months, using slide agglutination and stool culture techniques. The organisms isolated from infected patients were S. typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C. S. typhi was the most predominant among all the species and S. paratyphi was most occurring among the paratyphi. Pearson’s correlation coefficient (r = 518; P < 0.05) shows that significant serum titres were positively related to positive stool. For example, 671 patients (71.9%) had both significant serum titres (≥ 1: 160) and positive stool cultures. These findings point to the need for patients being advised to seek medical attention at the onset of any ailment and stool culture technique should be used in addition to the common Widal test used in the area for diagnosis.

Key word: Typhoid fever, Salmonella, Widal test, stool culture, Okpokwu LGA.

INTRODUCTION

Typhoid fever is an acute illness and a contagious infection caused by Salmonella typhi and paratyphoid fever is caused by Salmonella paratyphi A, B and C. Salmonella species are pathogenic bacterial parasites of man that can cause enteric fevers and food infection while S. paratyphi may be harboured by domestic animals, although, this is rare (Rose, 1983; Morbidity and Mortality Report, 1997; Cheesbrough, 2000).

Emmanuel et al. (2003) found that in Cameroon, typhoid fever infection was difficult to differentiate from other infections such as malaria because their symptoms often overlap. All cases of typhoid fever were traced to S. typhi and stool culture gave a better result than Widal test though Widal test indicated the presence of the organism.

Willke et al. (2002) worked on the diagnosis of typhoid fever in Turkey using Widal test, and made the following recommendations: (i) O and H agglutinin titres of ≥1/200 are significant in the diagnosis of typhoid infection (ii) although, the Widal test is an easy, inexpensive, and relatively non-invasive test that can be of diagnostic value in situations where cultures cannot be obtained, the results must be interpreted cautiously, as negative results do not exclude typhoid fever, and (iii) the Widal test performed 7 to 10 days after hospitalization is expected to give the most-reliable results with high specificity and sensitivity.

Widal test though controversial has some merits in areas where culture facilities are either poor or not available thus; misuse of Widal test should be strongly condemned (Onuigbo, 1991; Akinyemi et al., 2000). Where Widal testing is the norm, the use of rapid antigen screening directly from the stool of the suspected patient may be more useful (Olopoenia and Aprileona, 2000). Mohammad et al. (1998) also worked on diagnosis of typhoid fever using widal test and bacteriological culture results in Dhaka and found more men infected than women.

Smith et al. (2004) in his work in Nigeria found more
females to be infected with typhoid fever using Widal test. A higher number of infected patients had their serum agglutinated to the somatic antigen of \textit{S. typhi}.

There is little information available on typhoid fever in Benue State and the comparisons of the technique used in diagnosis of the disease in Okpokwu L.G.A of Benue State are unavailable. The purpose of this study was therefore to determine:

1. The prevalence of typhoid fever and paratyphoid fever in Okpokwu L.G.A using Widal test and stool culture techniques.
2. The comparison between the common Widal test techniques used with stool culture in diagnosis.
3. The association between the two techniques.

MATERIALS AND METHODS

Description of study area

Okpokwu Local Government Area of Benue State is one of the Local Government Areas in Idoma speaking area of the state and it is located in the Southern part of the state. It has a population of 176647 (2006, Census).

Scope of the study

The study covered hospitals in Okpoga, North and South Edumoga areas including Ugbokolo. Some are referral hospitals of the nearby communities.

Ethical consideration

Approval for this study was obtained from the Health Department, Okpokwu LGA. Confidentiality was maintained in accordance with standard medical practice. Consent of clinically suspected patients was sought.

Preparation of media

The appropriate media were weighed and prepared according to instructions of the manufacturers.

Sample collection

Over a period of nine (9) months, a total of one thousand four hundred and seventy- nine (1,479) blood samples and one thousand four hundred and seventy- nine (1,479) stool samples were collected from clinically suspected typhoid patients. Blood samples were collected from the arm; the arm just above the elbow was tied with a tourniquet in order to locate the vein. The site of collection was swabbed with cotton wool and spirit to make the area aseptic. With the aid of a sterile needle and syringe 3 ml of blood sample was collected from each patient. The blood was left to stand and clot to obtain the serum. Stool samples were collected in sterile containers previously shared to the patients. With the consent of the patients and parents of children, a questionnaire was used to collect bio data of patients and other information (name, age, sex, residential area, occupation, source of water used) and also to record result of the Widal test.

Microbiological analysis

Widal agglutination test

Serum was obtained from the blood sample. The test was performed using clean white tiles. Rapid slide titration method using commercial antigen suspension for somatic (O) and flagella (H) antigen of \textit{S. typhi} and \textit{S. paratyphi} A, B and C (Febrile antigen test kit from Antec Diagnostic Ltd by plasmatic Laboratory Products Ltd – United Kingdom).

Stool culture

Stool samples collected from patients were inoculated onto MacConkey agar. The culture plates were incubated at 37°C for 48 h. Absence of any lactose negative organism after 48 h was labelled negative. Colonies of lactose negative organisms which showed pale colour on MacConkey were inoculated into selenite F broth for enrichment cultures and subculture on Xylose Lysine, deoxycholate agar and \textit{Salmonella} - \textit{shigella} agar. The culture plates were read daily for 48 h. According to Cheesbrough (2000), \textit{Salmonella} shows black on all the colonies which indicate the presence of hydrogen sulphide (H₂S) except \textit{S. paratyphi} does not show black on Xylose-lysine deoxycholate agar and \textit{Salmonella- Shigella} agar. Colonies suspected to be \textit{Salmonella} were identified biochemically using the Gram stain, motility test, urease test, Lysine decarboxylase/ indole test, citrate utilization and confirmed serologically using polyvalent antigens, according to the methods of Cheesbrough (2000) and Willke et al. (2003).

Statistical analysis

All statistical analysis was carried out using SPSS version and Microsoft excel. Associations and correlations between variables were examined using chi-square and Pearson’s product moment correlation. Statistical significance was set at 0.05% confidence level.

RESULTS

Out of one thousand four hundred and seventy-nine
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Table 1. Frequency of significant and non significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Serum titre</th>
<th>Sample Test result (Widal)</th>
<th>No positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Significant serum titre (+ve)</td>
<td>919</td>
<td>62.2</td>
<td></td>
</tr>
<tr>
<td>Culture stool</td>
<td>Positive stool culture for S. typhi</td>
<td>757</td>
<td>51.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1479</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Significant serum titre: ≥1:160

Table 2. Biochemical characteristics of isolated serotype of S. typhi and S. paratyphi.

<table>
<thead>
<tr>
<th>Isolates (%)</th>
<th>Growth on SSA</th>
<th>Growth on MacConkey</th>
<th>Growth on XLD</th>
<th>Motility</th>
<th>Gram stain</th>
<th>Urease</th>
<th>LDC</th>
<th>Indole</th>
<th>Citrate utilisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>752(51.2)</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

XLD-xylene deoxycholate agar; SSA-Salmonella-shigella agar.

Table 3. Occurrence of significant serum titre among S. typhi positive stool samples.

<table>
<thead>
<tr>
<th>Stool samples</th>
<th>Occurrence (%) significant serum titre +ve for Salmonella typhi</th>
<th>Occurrence (%) significant serum titre +ve for Salmonella typhi</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive stool</td>
<td>661(71.9)</td>
<td>96(17.1)</td>
<td>757(51.2)</td>
</tr>
<tr>
<td>Negative stool</td>
<td>258(28.1)</td>
<td>464(82.9)</td>
<td>722(48.8)</td>
</tr>
</tbody>
</table>

χ² = 417.958; P<0.05; significant serum titre: ≥ 1:160.

Table 4. correlation coefficients of variables in respect to seasonal changes of salmonella infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>X₁</th>
<th>X₂</th>
<th>X₃</th>
<th>X₄</th>
<th>X₅</th>
<th>X₆</th>
<th>X₇</th>
<th>X₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₂</td>
<td>0.015</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₃</td>
<td>0.366**</td>
<td>0.718**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₄</td>
<td>0.702*</td>
<td>0.021**</td>
<td>0.089**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₅</td>
<td>0.640**</td>
<td>0.440**</td>
<td>0.319**</td>
<td>0.518**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₆</td>
<td>0.795**</td>
<td>0.068**</td>
<td>0.361**</td>
<td>0.520**</td>
<td>0.545**</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₇</td>
<td>0.052**</td>
<td>0.808**</td>
<td>0.635**</td>
<td>0.000</td>
<td>0.361**</td>
<td>0.105**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>X₈</td>
<td>0.109**</td>
<td>0.157**</td>
<td>0.104**</td>
<td>0.039**</td>
<td>0.171**</td>
<td>0.06**</td>
<td>0.225**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

** = Correlation is Significant at 0.05%, X₁ Status of Salmonella typhi, X₂ Status of S. paratyphi, X₃ Co-infection status, X₄ Stool culture, X₅ Serum titre of patients, X₆ Serotypes of S. typhi, X₇ Serotypes of Salmonella paratyphi and X₈ Seasonal change.

(1,479) blood and 1479 stool samples examined, nine hundred and nineteen (919) (62%) had significant serum titre and seven hundred and fifty-seven (757) (51.2%) showed positive stool cultures indicative of infection by S. typhi and S. paratyphi (Table 1). Table 2 summarizes the number of isolates, identification and characteristics of S. typhi, S. paratyphi A, B and C.

Positive S. typhi stool culture was significantly associated with significant serum titre (χ² = 417; p < 0.05). Out of the nine hundred and nineteen (919) patients that had significant serum titre, 71.9% had S. typhi positive stool culture, whereas, only 17.1% of those without significant serum titre had positive stool culture (Table 3). The correlation coefficients of some of the variables were studied. Significant serum titres and positive stool culture were significantly correlated (r = 518**; P< 0.05) (Table 4).

DISCUSSION

The results of this study showed that out of the 1,479 clinically suspected typhoid patient as much as 661 (71.9%) had significant serum titres and positive stool cultures. Widal test is the only technique used in the local...
Government area and it is not uncommon in other parts of Benue state and Nigeria at large. This finding agrees with those of Emmanuel et al. (2003), Brush et al. (2006) and Jombo et al. (2007) who worked in Cameroun, Asia, Africa, Latin America and Nigeria respectively.

Infected patients sera agglutinated more with the somatic antigen of S. typhi than the flagella antigen. This finding resembles those of Mbuh et al. (2003), Emmanuel et al. (2003), Smith et al. (2004) and Chart et al. (2007). Among the S. paratyphi, S. paratyphi A was commonest. This microorganism has been reported to cause typhoid fever in some places. For example, Ochiai et al. (2005) found that S. paratyphoid A caused typhoid fever in Asia, China and India. Widal test used for diagnosis of typhoid fever though controversial according to previous studies, seem useful in diagnosis and gives 75.2% sensitivity to typhoid fever (Cheesbrough, 2000) which relates closely with the findings of this study. Stool culture also shows to be a very useful tool of diagnosis of Salmonella infection in the study area as it has earlier been reported to give about 80% accurate result after 3 weeks of infection (Cheesbrough, 2000). This is because most of these people do not seek medical attention on time. Generally, the number of those with significant serum titres was more than those with positive stool cultures. This finding agrees with that of Emmanuel et al. (2003) and Mbuh et al. (2003) though using blood culture. This may be due to individuals host immune responses which may be stimulated in non-typhoid febrile illnesses (Cheesbrough, 2000).

Conclusion

In conclusion, from the total number of clinically suspected typhoid patients, significant serum titre is associated with positive stool culture. Widal test used for diagnosis of typhoid fever though controversial seems to be useful especially where there are no facilities for culture. Stool culture may be better for the diagnosis of patients after 3 weeks of infection with typhoid fever especially in a place like Okpokwu Local Govt Area where they do not go to the hospital on time. The following therefore is recommended;

- Using Widal test as method of diagnosis of typhoid fever should not be totally discouraged but that either blood or stool culture should be encouraged in addition.
- Patients should be advised to go to the hospital at the onset of any infection especially when the body temperature becomes very high.
- Typhoid test should be conducted at intervals and the chemotherapy frequently administered.

Further work should be done in the comparison of Widal test, stool and blood culture used as diagnostic tools in typhoid fever.

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REFERENCES


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