

## Original article

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# Clinical and laboratory features of typhoid fever in childhood

## Focus on relationship between prior antibiotic administration with blood culture, Widal and Typhidot results

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**ABSTRACT** The aims of the study were to review the clinical features of patient with suspected typhoid fever, to obtain features of the blood culture results, Widal, Typhidot tests and to identify the relationship between/prior antibiotic administration with laboratory findings. The study had been conducted since January 1, 1999 till January 31, 2000 with inclusion criteria (1) age of patient was 3 to 14 years, (2) patient with diagnosis of suspected typhoid fever and (3) gave consent to participate in the present study. Thirty-six patients were eligible for this study and the result showed that typhoid fever was encountered more commonly in girls with the most prevalent onset was in age 5 to 9 years and duration of fever was less than 8 days. Gastrointestinal tract disturbance was the most frequent complain after fever. Most patient showed negative results in blood culture examination and Widal test, and gave positive results in Typhidot/Typhidot M test as well. Antibiotic administration tended to influence blood culture and Widal test as to need consideration in evaluation of laboratory results. However, a conclusion still could not be drawn definitively that further study with adequate sample number and positive blood culture as criteria inclusion was needed. [Paediatr Indones 2001;41:149-154]

**Keywords:** typhoid fever, blood culture, Typhidot

TYPHOID FEVER IS AN ACUTE SYSTEMIC DISEASE CAUSED BY infection of *Salmonella typhi*. Up to now typhoid fever is still a community health problem and relates to poor sanitation, especially in developing countries.<sup>1</sup> The clinical features of typhoid fever in childhood are often not specific, even asymptomatic. Therefore, to establish the diagnosis, reliable laboratory investigations are needed.<sup>2</sup> Actually, microorganism culture is highly diagnostic, but it may take as long as before the result

is obtained.<sup>2,3</sup> The value of serologic test (Widal test) is a matter of debate, and interpretation of Widal test should be done carefully because of many affecting factors.<sup>4</sup> Typhidot examination is a method of *Dot Enzyme Immunosorbent Assay* using specific protein (only owned by *S. typhi* organism). This method provides chance to assess serodiagnostic test in patient with typhoid fever.<sup>5</sup> Most patients referred to hospital have received prior antibiotic that might have influence on the blood culture, Typhidot and Widal results. The aims of this study were to review the clinical features of suspected typhoid fever and to obtain the features of culture blood, Widal, Typhidot results and to identify the relationship between antibiotic administration with laboratory results.

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## Methods

This study was carried out prospectively in Department of Child Health, Medical School University of Indonesia Cipto Mangunkusumo Hospital, Jakarta. Samples were taken from all cases with suspected typhoid fever hospitalized in Department of Child Health, Medical School University of Indonesia Cipto Mangunkusumo Hospital during period of January 1, 1999 until January 31, 2000. Inclusion criteria included (1) age of patient was 3 to 14 years, (2) patients with diagnosis of suspected typhoid fever and (3) gave consent to be included in the study. In patients meeting inclusion criteria peripheral blood, blood culture, Widal, Typhidot and Typhidot M tests were done.

Blood culture was done using BACT Alert 120.<sup>6</sup> The Widal tests was done using Burrough Wellcome agglutinable suspensions of *S.typhi*, *S. paratyphi A*, *S. paratyphi B* and *S. paratyphi C*. Serial dilutions of the subjects serum of 1:20, 1:40, 1:80 and 1:160 in 0,25% phenol saline were tested for agglutinins to these four species of Salmonella. Where a 1:160 dilution gave a positive result, further dilutions were tested. The O agglutinins were tested by adding 0,5 ml of the O-agglutinable suspension to each tube of diluted serum, incubating for 2 hr at 37°C, and the refrigerating overnight. The tubes were read after standing at room temperature for 2 hr. The H agglutinins were tested by adding 0,5 ml of the H agglutinable suspension to the tubes of diluted serum and incubating for 2 hr at 50°C, after which they were read.<sup>4</sup>

Serodiagnosis was also performed using both the Typhidot and Typhidot M. In the case of the Typhidot test, 1:100 dilutions of patient serum were applied to standard aliquots of 0,3 mg purified OMP dotted on to nitrocellulose strips. After 1 hour, horseradish peroxidase conjugated antiserum to human IgG or IgM added. The presence of antigen-antibody complexes was assessed visually from the resultant color change in comparison to that from positive control sera. Because only one dilution was used, a positive Typhidot test was taken as an IgG and/or IgM titer of at least 1:100. In the case of Typhidot M test, diluted sera were first added to a buffered inactivation solution containing anti human IgG. Other procedures were similar to those of the original Typhidot test except that inactivated control sera were used for visual comparison.<sup>7,8</sup>

Descriptive data were presented in text and tables. Association between previous antibiotic administration with blood culture, Widal and Typhidot tests was sought.

## Results

### Characteristics of the patients

Out of 36 patients under study, 14 boys (39%) and girls of 22 (61%) were found. Patients were most commonly in of 5-9 year age group, as many as 23 (64%) cases with mean age of 8.4 years and standard deviation was 2.6 years (See Table 1).

TABLE 1. DISTRIBUTION OF AGE AND SEX

Age group (year)	Male	Female	Total
< 5	1	1	2
5 - 9	11	12	23
> 10	2	9	11
Total	14	32	36

Most cases were admitted after developing fever of less than 8 days (78%), the shortest was 5 days and the longest was 14 days. The mean duration of fever at home was 7.1 days with standard deviation of 2.2 days. The second most common complain was gastrointestinal tract disturbance.

In addition to symptom of fever, delirium, nausea, vomiting, diarrhea, obstipation, loss of appetite and abdominal pain symptoms were also seen. Decrease in consciousness was found only in one patient. On physical examination, typhoid tongue was observed in five patients and hepatomegaly encountered in 14 (39%) patients (see Table 2).

On peripheral blood features, anemia was found in 8% patients (age  $\leq$  6 years) and 42% patients (age  $>$  6 years), leukopenia (36%) patients, lymphocytosis (23%) patients (see Table 3).

Blood cultures were positive in 10 (24%) patients. There were 4 (11%) patients with positive Widal tests, 26 (72%) patients with positive Typhidot and 29 (81%) patients with positive Typhidot M (see Table 4).

**TABLE 2. CLINICAL SYMPTOMS OF 36 PATIENTS WITH DIAGNOSIS OF SUSPECTED TYPHOID FEVER**

Clinical symptoms	n	%
Fever	36	100
Delirium	16	44
Decreased consciousness	1	3
Nausea	25	69
Vomiting	16	44
Diarrhea	9	24
Obstipation	28	78
Anorexia	21	58
Abdominal pain	10	28
Flatulence	4	11
Typhoid tongue	5	14
Hepatomegaly	14	39
Splenomegaly	2	56

**TABLE 3. FEATURES OF PERIPHERAL BLOOD STUDIES**

Peripheral blood features	%
Anemia	
≤ 6 years	8
> 6 years	42
Leukopenia	36
Lymphocytosis	23
Increased ESR	74

**TABLE 4. FEATURES OF WIDAL, TYPHIDOT, TYPHIDOT M SEROLOGIES**

Studies	Number	Percentage
Widal Test		
Positive	4	11
Negative	32	89
Typhidot		
Positive		
IgM+ and IgG -	2	6
IgM+ and IgG+	24	67
Negative		
IgM- and IgG+	6	17
IgM- and IgG -	4	11
Typhidot M		
Positive.	29	81
Negative	7	19
Blood culture		
Positive	10	28
Negative	26	72
Total	36	100

### Association between previous antibiotic administration with laboratory results

Out of 36 patients there were 10 patients with positive blood cultures in which seven patients have received antibiotic previously. Negative blood cultures were encountered in 26 patients, most of which (24 patients) have been given antibiotic. Table 5 shows association between previous antibiotic administration with blood culture results.

**TABLE 5. ASSOCIATION BETWEEN PREVIOUS ANTIBIOTIC ADMINISTRATION WITH BLOOD CULTURE RESULTS**

Previous antibiotic administration	Blood culture		Total
	Positive	Negative	
Yes	3	2	5
No	7	24	31

*Risk ratio* : 5,14 (95% CI: 0.71-37.1)

*Risk ratio* of 5.14 meant that those received antibiotic would have probably of negative results in blood culture as 5.14 times compared to those not received antibiotic. However, the confidence interval included one number due to the small sample number.

Among 36 patients there were four patients with positive Widal test results in which three patients have received prior antibiotic. Negative Widal test results were observed in 32 cases in which most of them (28 patients) have been given antibiotic. Table 6 shows association between previous antibiotic administration with Widal test results.

**TABLE 6. ASSOCIATION BETWEEN PREVIOUS ANTIBIOTIC ADMINISTRATION AND WIDAL TEST RESULTS**

Previous antibiotic Adnstration	Widal Test		Total
	Positive	Negative	
No	1	4	5
Yes	3	28	31
Total	4	32	36

*Risk ratio* : 2,3 (95% CI: 0.193-28.3)

Risk ratio of 2.33 was encountered that meant those who have received antibiotic would give negative Widal results more frequently as many as 2.33 times compared to those upon whom received antibiotics were not given. Confidence interval of this risk ratio included one number because of the small sample number.

Out of 36 patients, 26 patients gave positive Typhidot results in which 23 patients have received antibiotic previously. Negative Typhidot results were observed in 10 patients in whom eight patients have received antibiotic. Table 7 depicts association between previous antibiotic administration with Typhidot test results.

**TABLE 7. ASSOCIATION OF PREVIOUS ANTIBIOTIC ADMINISTRATION WITH TYPHIDOT RESULTS**

Previous antibiotic administration	Typhidot		Total
	Negative	Positive	
Yes	2	3	5
No	8	23	31
Total	10	26	36

Risk ratio : 1.92 (95% CI: 0.27-13.63)

Risk ratio of 1.92 was found in those have received antibiotic who would show positive results in Typhidot test of 1.92 times greater than those who have not received antibiotic. However, this confidence interval included one number due to the small sample number.

## Discussion

### Characteristics of patients

Out of 36 patients studied outnumbered females (61%) were greater in number than males (39%). In the literatures it was said that there were no difference between males and females.<sup>9</sup> In the present study, most of the patients (23 patients; 64%) were found to be aged 5 to 9 years, only 2 patients aged under 5 years. This was in agreement with literatures stating that typhoid fever was more commonly contracted by schoolchildren, and only the minority occurred in age under 5 years. The incidence rate of typhoid fever in underfives was low because those who aged under 5

years rarely consume food/drink contaminated by *Salmonella typhi*.<sup>9</sup>

Usually, typhoid fever in childhood provides mild clinical features or even asymptomatic. Most patients (78%) developed fever at home of less than 8 days (See Table 3). In the study carried out by Rivai et al,<sup>1</sup> mean fever before hospitalization of 9,2 days among 106 patients under study was found.

The second most common complain after fever in the present study was gastrointestinal tract disturbance. In this study, diarrhea was observed in 9 (24%) patients, constipation in 28 (78%) patients, nausea in 35 (69%) patients, vomitus in 16 (44%) patients, epigastric pain in 10 (28%) patients (See Table 2). These were in accordance with study by Nathin and Harun in the year 1985-1988, obstipation (52.9%) was more frequently found than diarrhea (35.3%).<sup>10</sup> In the previous year 1978-1979 Sumarmo et al, also found constipation (27.5%) was more common than diarrhea (18.3%).<sup>8</sup> Rivai et al,<sup>1</sup> their study observed diarrhea (39.47%) was more frequent than constipation (15.79%).

Delirium in this study was seen in 16 (44%) patients, disturbed consciousness of somnolence only found in one patient. Consciousness disturbance of apathy was not seen in cases under study. This condition was much different from report by Rivai et al observing 31.6% of cases being studied were accompanied by apathy.<sup>1</sup>

Features of peripheral blood in typhoid fever were generally characterized by leukopenia with relative lymphocytosis and disappearance of eosinophil. In the study, leukopenia was seen in 13 (36%) out of 35 patients being examined. Study by Rivai et al,<sup>1</sup> found leukopenia in 52% of patients. Whereas Soelistyowati et al,<sup>9</sup> in their study observed leukopenia in 98% patients. In children aged  $\leq 6$  years, anemia was encountered in 3 (8%) out of 35 patients under investigation, whereas in those aged  $> 6$  years, anemia was seen in 12 (42%) out 35 study patients.

In the present study there were only 10 (28%) patients with positive blood cultures. Sumarmo et al,<sup>8</sup> in Department of Child Health, Medical School University of Indonesia Cipto Mangunkusumo Hospital, Jakarta reported that in the year 1975-1976 among 118 cases that were clinically with typhoid fever, 40.7% blood cultures were positive. In the same hospital in 1978-1979, 22.5% of positive cultures out of 120 pa-

tients in the period of 5 years (1990 up to 1994) of 232 cases with typhoid fever upon whom cultures could be performed only 36.2% giving positive results.<sup>11</sup>

There are several factors influencing blood culture results, such as the time of blood sampling, blood amount and prior antibiotic administration.<sup>11,12</sup> In terms of blood drawing time, in 28 (78 %) patients, blood drawing was done on the first week of illness, the remaining 8 (22%) patients was on the second week of illness. According to literatures positive blood cultures were found in a 75-80% of patients in the first week of illness, while in the end of third week, positive blood cultures in 10% of patients.<sup>3,9</sup> Watson in his work observed the positivity of blood cultures in the first week of fever was 72%, increased to 91% in the second week and in the third week declined to 83%.<sup>13,14</sup>

To increase the positivity of blood culture results the amount of blood that should drawn as specimen of 5 ml was advisable, and the blood better directly put into gall media.<sup>12</sup> According to Watson for blood culture 5 to 10 ml blood was needed.<sup>14</sup> In other literature it is said that the required blood amount for blood culture was 3-5 ml.<sup>12</sup>

In the present study, one kind of dilution was used to express antibody O titer, namely dilution of 1:80 with its multiplication (1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560). Widal test results of the study considered positive when antibody titer O  $\geq$  1/160 in single test or increase of antibody titer O of 4 times with interval of 7 -10 days or H titer  $\geq$  1/640.<sup>15</sup> Using that standard 4 (11.1%) cases were found to positive in Widal test. These results were lower in comparison with other study finding positivity of Widal test in 13.7% of cases.<sup>16</sup>

Nevertheless, it is should be noted that many factors affecting results Widal test, among others are history of prior antibiotic treatment. It is stated, in literature, that antibiotic administration would suppress antibody development. It is mentioned that long-term administration of chloramphenicol will cause bone marrow depression as to compromise immunity.<sup>17</sup>

Another factor affecting Widal test results was the time of blood drawing. In this

study most (77.8%) patients presented in the first week of illness. It is mentioned, in literature, that in the first week, approximately 20% of patients show the presence of antibody. Subsequently, an increase

will occur progressively and will reach its peak in the fourth week of illness. Therefore, Widal testing should be carried out after the first week and done at least twice with interval of 7-10 days to assess the presence of increase in antibody titer.<sup>16</sup>

In the study, 26 out of 36 patients provided positive results in Typhidot examination. Results of Typhidot test considered positive if IgM was positive regardless of whether IgG was positive or negative. Study with Typhidot M found more cases showing positive results as many as 29 cases. This could be explained that Typhidot kit might find *masking effect* in which IgM was masked by such high IgG levels so that IgM result was negative and IgG was positive. In this condition, IgG was likely to come from past infection or in the initial stage of reinfection.<sup>5,18</sup>

### Association of previous antibiotic administration with laboratory results

Table 5 shows that patients who have received antibiotic had the opportunities to give negative results in blood culture as many as five times compared to patients who have not received antibiotic. This was in agreement with literatures, that antibiotic treatment would kill organisms in blood circulation several hours after administration, whereas organisms in bone marrow were harder to kill.<sup>19</sup> Blood culture testings, therefore, should be carried out before antibiotic administration because 1-2 days after antibiotic administration the organisms have been difficult to identify in blood.<sup>20</sup> Gilman found positive blood cultures in 80% of patients who had not received antibiotic, whereas in patients upon whom antibiotics had been given, blood cultures were positive in 40% patients and bone marrow culture in 90% of patients. Bachtin in his study observed positive blood cultures in 8 out of 17 patients not previously treated with chloramphenicol, while in patients received chloramphenicol, positive blood cultures were seen in 5 among 17 patients.<sup>21</sup>

In Table 6 it can be seen that patients who have received antibiotic showed the opportunities have negative results in Widal test as twice greater as of those who have not received antibiotic.

Many factors are known affecting Widal test results including antibiotic administration. This antibiotic administration would cause many organisms *S.typhi* die as depress agglutinin formation due to decreased

stimulation by antigen.<sup>22</sup> In the opinion of Schroeder<sup>23</sup> failure in increase of titer  $\geq 4$  times was related to antibiotic given (especially chloramphenicol) prior to repeat studies. Table 7 depicts patients that received antibiotic get the opportunities to provide positive results in Typhidot test as many as 1.92 times in comparison to those who have not received antibiotic.

In the present study, fever was encountered more commonly in girls with the most prevalent onset was in age 5 to 9 years and duration of fever was less than 8 days. Gastrointestinal tract disturbance was most frequent complaint after fever. Most patients showed negative results in blood culture examination and widal test, and gave positive results in Typhidot/Typhidot M test as well. Administration of antibiotic tended to affect laboratory results namely by reducing the proportion of positive blood cultures and Widal findings, so that it should be noted that previous antibiotic administration should be considered in evaluation of laboratory results. However, a conclusion still could not be drawn definitively that further study with adequate of sample size and inclusion criteria incorporating positive blood cultures was needed.

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