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Evaluation of Dri-Dot OMPs *Salmonella Typhi* in Suspected Typhoid Fever Patients as an Immunodiagnostic Kit

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Abstract: Background. Several studies have identified a protein of OMP S. Typhi. The protein is highly immunogenic and can be recognize whole cells of S. Typhi. The aims of the study was to evaluate the Dri-dot OMPs immunoreactivity in typhoid fever suspected patients. Method. Samples obtained from Dr. Wahidin Sudirohusodo Hospital, lbnu Sina Hospital, and Haji Hospital of Makassar from February to November 2014. Their sensitivity and specificity were evaluated against blood culture method as the gold standard. For each of the two tests, sensitivity, specificity, positive and negative predictive values were calculated using standard formulae. Results. A total of 15 of suspected typhoid fever samples examined for culture, lateral flow and Dri-dot OMPs. Examination of blood culture method showed that 3 of 15 (20%) patients had a blood culture positive for S. typhi. Dri-dot OMPs were positive in 13 (86.7%) serum samples. Fourteen (93.3%) serum samples were positive for lateral flow examination. Thirteen samples were positive for both Dri-dot and lateral flow. One sample was negative for both lateral flow and Dri-dot. One sample was positive for Dri-dot but negative for lateral flow. Conclusion. We conclude that there was no difference between Dri-dot OMPs, lateral flow and widal tests. Dri-dot could be of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever.

Keywords: Salmonella Typhi, Typhoid Fever, Lateral Flow, Dri-Dot OMPs

1. Background

Typhoid (enteric) fever is still a common disease in many developing countries but current diagnostic tests are inadequate [1]. Typhoid fever is responsible for an estimated yearly burden of 16,591,000 cases and 580,000 deaths worldwide. The greatest burden of illness occurs in Asia, where 13,310,000 cases and 440,000 deaths occur annually [2]. Typhoid fever only found in its human host [3]. In the regions where enteric fever is common, clinical diagnosis of typhoid fever is inadequate, as the symptoms it causes are nonspecific and overlap with those of other febrile illness, such as vector-borne malaria, dengue fever and rickettsioses as well as environmentally transmitted leptospirosis and melioidosis [4, 5].

The clinical signs of uncomplicated typhoid fever are nonspecific, and an accurate diagnosis on clinical grounds alone is difficult. In developing countries most serotype Typhi infections are diagnosed purely on clinical grounds and treated presumptively. As a result, the diagnosis may be delayed or missed while other febrile illnesses are considered, and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial therapy [6]. Although a definitive diagnosis can be made by isolation of *S. typhi* from blood or bone marrow, but in endemic areas such as Indonesia, bacterial culture facilities are often unavailable [7].

Detection of serum antibodies using the Widal test, which is currently the standard serological method for typhoid diagnosis. However, this method is relatively low specificities [8]. Need some efforts to develop faster and more sensitive and specific serological assays for the diagnosis of typhoid fever.

Previous study, has been addressed to the role of the outer membrane proteins (OMPs) of gram-negative bacteria in the induction of specific immunity. The results of this study indicate the usefulness of OMPs in the induction of active immunity against *S. typhi* in mice [9]. Outer membrane proteins was found to be immunogenic and capable of stimulating both humoral mucosal and systemic immunity. Outer membrane proteins was also able to stimulate cellular immune responses [10]. Because of its ability to stimulate the immune response and protection against bacterial adhesion, we investigated OMPs as a diagnostic tool for typhoid fever.

2. Methods

2.1. Isolation of S. Typhi Strain

Virulent *S. typhi* was originally isolated from a patient with typhoid fever. Five mL of patient blood was taken aseptically and then inserted into the BACTEC transport medium and incubated at 37°C for 24-48 hours. This culture were then incubated on selective medium (MacConkey) at 37°C for 18-24 hours. Identification of *S. typhi* was carried out by its colony characteristic on culture medium. Biochemical and microscopic tests (Gram's staining) were performed to confirm the presence of *S. thypi* [11, 12].

2.2. Isolation of OMPs

Salmonella OMPs were extracted by following protocol described by S. Kim *et al* (2006) [13]. In brief, 6-8 ose culture of *S. typhi* was added to BHIB 1 ml of medium and incubated at 37°C for 24 hours. Furthermore, the cell cultures were centrifuged at 15,000g for 20 min at 4°C. The pellets then added with 10mM 11 Tris-HCl (pH 8) and sonicated on ice using a sonicator for 4 times for 5 seconds [14], centrifuged at 15,000 g for 1 h at 4°C. Returned pellets were separated and added with 10mL of 10mM 1-1 Tris-HCl (pH 8) and sarcosyl to reach a final concentration of 1.5% (v / v). After 20 min at room temperature, the outer membranes were collected by centrifugation at 15,000 g for 90 min at 4°C. We use the outer membranes protein to activate the latex beads (Dri-dot OMPs).

2.3. Evaluation of Diagnostic Accuracy of the Rapid Kits

The results from the two rapid kits were evaluated against blood culture method as the gold standard. For each of the two tests, sensitivity, specificity, positive and negative predictive values were calculated using standard formulae. Sensitivity, which is the percentage of positive individuals correctly identified as such, was calculated using the formula: Sensitivity = number of true positives (TP)/(number of TP + number of false negatives (FN)) × 100%. Specificity, which is the percentage of negative individuals correctly identified as such, was calculated with the formula: Specificity = number of true negatives (TN)/(number of TN+ number of false positives (FP)) × 100%. Positive predictive value (PPV), the proportion of positive test results that are truly positive, was calculated using the formula: PPV = TP/(TP + FP) × 100%, and the negative predictive value (NPV), which is the proportion of negative test results that are truly negative was calculated using the formula: NPV=TN/(TN+ FN) × 100%.[13] Patients in this study had symptoms and signs of typhoid, which included persistent and high fever (>38°C oral temperature), chills malaise, headache, sore throat, cough and sometimes abdominal pain and constipation or diarrhea. Fifteen samples of suspected typhoid fever were examined using Dri-dot OMPs, lateral flow test and bacterial culture.

2.4. Ethical Approval and Informed Consent

Institutional and ethical permission to carry out the study was obtained from the Faculty of Medicine Hasanuddin University Makassar Indonesia. Adult participants and parents/guardians of sick children provided by informed consent before blood samples were collected.

3. Results

Table 1. *Blood culture in patients suspected of typhoid fever.*

		N	%
Culture	Positive	3	20
	Negative	12	80
	Total	15	100

Table 2. *Dri-dot OMPs results in serum samples of typhoid fever patients.*

		N	%
Dri-dot OMPs	Positive	13	86.7
	Negative	2	13.3
	Total	15	100

Table 3. *Lateral flow results in serum samples of typhoid fever patients.*

		N	%
Lateral Flow	Positive	14	93.3
	Negative	1	6.7
	Total	15	100

Table 4. *Sera from typhoid fever patients examined by Dri-dot OMPs and Lateral flow.*

		Dri-dot OMPs		
		Positive	**Negative**	**Total**
Lateral Flow	Positive	13	1	14
	Negative	0	1	1
	Total	13	2	15

A total of 15 samples examine for culture, lateral flow and Dri-dot OMPs. Examination of blood culture method showed that 3 of 15 (20%) patients had a blood culture positive for *S. typhi*. The results of the culture examination are presented in Table 1. Dri-dot OMPs were positive in 13 serum samples.

The results of the Dri-dot OMPs examination are presented in Table 2. Fourteen serum samples were positive for lateral flow examination (Table 3). Thirteen samples were positive for both Dri-dot OMPs and lateral flow. One sample was negative for both lateral flow and Dri-dot OMPs. One sample was positive for Dri-dot OMPs but negative for lateral flow as shown in Table 4.

Table 5. *Comparative evaluation of Dri-dot OMPs and Lateral flow (if blood culture as a gold standard).*

	No. of positive among positive culture cases (n = 3)	No. of positive among negative culture cases (n = 12)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Dri-dot OMPs	3 (100%)	10 (83.3%)	100%	16.7%	23.1%	100%
Lateral Flow	3 (100%)	11 (91.7%)	100%	8.3%	21.4%	100%

4. Discussion

This study was conducted during the period of February to November 2014. As many as 15 patients clinically suspected typhoid fever were included in this study. Samples were obtained from Ibnu Sina Makassar Hospital, Dr. Wahidin Sudirohusodo Hospital, Akademis Hospital and Haji Makassar Hospital.

Typhoid fever often causes severe mental clouding of consciousness and includes headache, anorexia and congestion of mucous membrane which result from toxic effects of *S. typhi* endotoxin. The definitive diagnosis of typhoid fever requires the isolation of organism from blood, bone marrow or rose spots [14-16]. Blood culture is considered as gold standard in the diagnosis of typhoid fever but it is a slow process, time consuming, its use is limited in early cases of fever, it becomes rapidly negative with administration of antibiotics and available in higher laboratories [14, 17].

The purpose of this study was to develop rapid diagnostic for typhoid fever using Dri-dot OMPs. We compared Dri-dot OMPs results with blood culture and lateral flow in suspected typhoid fever patients. In this study, among 15 widal positive serum samples we found only 3 samples positive by culture therefore, culture was positive in 20% of all cases with a clinical suspected of typhoid fever. Possible causes of this negative culture results in this study was the fact that most of the patients are likely to have taken antibiotics before seeking medical care at the hospital. It is commonly in developing country including Indonesia. Blood culture is highly sensitive to the presence of antibiotics in the sample [18]. We used blood culture-positive patients as a gold standard, because bone marrow culture as a gold standard is difficult to perform. The results presented here showed that all culture positive samples were positive on lateral flow and Dri-dot OMPs.

When using blood culture as gold standard, sensitivity of lateral flow and Dri-dot OMPs was 100% but specificity very low were 8.3% and 16.7% respectively. Positive predictive value of Lateral flow was 21.4% and positive predictive value of Dri-dot OMPs was 23.1%. Lateral flow and Dri-dot OMPs had 100% of negative predictive value. The results presented here show that Dri-dot OMPs and lateral flow tests for the detection of IgM antibodies to serotype Typhi LPS perform no differences than the Widal test.

The antibody response to *S. typhi* as well as other infectious agent provides useful diagnostic markers of the infection caused in a host. Pathogen-specific IgM antibodies appear quickly within weeks after the infection but disappear soon afterward [19]. These tests could be of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever but are culture negative or in regions where bacterial culturing facilities are not available [20].

Early diagnosis and complete treatments reduce the complications in typhoid fever [21]. In this study, we designed a new and rapid diagnostic method based on latex agglutination test (Dri-dot) using OMPs antigen to detect antibody anti-OMP in serum samples.

5. Conclusion

We conclude that there was no difference between Dri-dot OMPs, lateral flow and widal tests. Dri-dot OMPs could be of use for the diagnosis of typhoid fever in patients who have clinical typhoid fever.

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