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Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia

M. HUSSEIN GASEM, HENK L. SMITS*, MARGA G. A. GORIS* and WIL M. V. DOLMANS†

*Department of Medicine, Dr Kariadi Hospital and Faculty of Medicine, Diponegoro University, Semarang, Indonesia, *Department of Biomedical Research, Royal Tropical Institute, Amsterdam and †Department of Medicine, University Medical Center St Radboud, Nijmegen, The Netherlands*

To support the clinical diagnosis of typhoid fever in Indonesia, where most hospitals and health centres have no facilities for culture, a rapid dipstick assay for the detection of *Salmonella typhi*-specific IgM antibodies was evaluated on serum samples from 127 patients clinically suspected of having typhoid fever. In a single blood sample collected on admission to hospital, the sensitivity of the dipstick assay was 69.8% when compared with bone marrow culture and 86.5% when compared with blood culture. The specificity as calculated for the group of patients with suspected typhoid fever but a negative culture result was calculated to be 88.9%. Of 80 patients with febrile illnesses other than typhoid fever, reactivity was observed in only three patients with dengue haemorrhagic fever. The assay uses stabilised components that can be stored outside the refrigerator, does not require special equipment, and may be of use in remote health facilities that have no culture facilities.

Introduction

Typhoid fever is an important public health problem in the tropics and developing countries like Indonesia [1]. The disease often does not show a specific clinical picture, especially early in the disease, and can be confused with other febrile illnesses such as dengue fever, malaria and leptospirosis, which are often prevalent in the same areas. The clinical diagnosis of typhoid fever requires confirmation by laboratory testing. Culture of either blood or bone marrow is time-consuming and laborious, and culture facilities are rarely available in hospitals in endemic areas. Furthermore, blood culture has limited sensitivity, particularly after previous antibiotic treatment. The sensitivity of bone marrow culture is up to 95% and is less affected by antibiotic treatment [2–4].

In Indonesia, where most hospitals and health centres in rural areas have no laboratory facilities, the diagnosis of typhoid fever is mostly based on clinical grounds, sometimes supported by the Widal test. In Central Java, one of the most developed parts of the country with >31 million inhabitants, only 11 of 115

state and private general hospitals have culture facilities. The Widal test is widely used in Indonesia, but is of limited value and difficult to interpret in an area where salmonella infections are endemic, especially if only a single blood sample is tested [5–7]. Therefore, a simple and rapid non-culture assay for the diagnosis of typhoid fever would be of great benefit in circumstances where more sophisticated laboratory support is not in place. In this study, a newly developed rapid dipstick assay was applied for the detection of *S. typhi*-specific IgM antibodies in serum and the results were compared with blood and bone marrow culture.

Materials and methods

Patient population

The study included 127 patients clinically suspected of having typhoid fever admitted to four hospitals in Semarang, Indonesia: 61 patients were admitted to the Dr Kariadi Hospital and 66 patients to three district hospitals (the Semarang Municipal Hospital, the St Elizabeth Hospital and the Telogorejo Hospital). For each patient, data were recorded regarding duration of fever before admission, history of prior antibiotic use and complications during admission. Eighty patients with other febrile illnesses due to dengue fever or dengue haemorrhagic fever ($n = 40$), leptospirosis ($n = 11$), urinary tract infection ($n = 10$), pneumonia

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Corresponding author: Dr M. H. Gasem (e-mail: sultana@indosat.net.id).

(n = 8), falciparum malaria (n = 4), tuberculous meningitis (n = 2), acute pharyngitis (n = 2), tetanus (n = 2) and measles (n = 1) served as negative controls and to test for cross-reactivity. Samples for culture and for the dipstick assay were collected on the day of admission to hospital. A single serum sample was collected from each patient.

Culture

For patients admitted to the Dr Kariadi Hospital, both bone marrow and blood culture were performed; in the remaining hospitals only blood culture was done. Cultures were performed with the BACTEC 9120 system (Becton Dickinson, NJ, USA). For blood culture, 8–10 ml blood was used; for bone marrow culture, 1–2 ml of bone marrow aspirate were used.

Dipstick assay

The dipstick consists of a strip of nitrocellulose membrane containing a 2-mm wide line of immobilised antigen as a detection band and a separate line of immobilised anti-human IgM antibody as the reagent control. The nitrocellulose strip was attached to a rigid backing. The antigen was prepared from a culture of a recent isolate of *S. typhi* from Indonesia. The culture was grown in LB broth and the antigen was prepared by heating a washed and 30× concentrated bacterial cell suspension of a 3-day-old well-grown culture for 30 min at 95°C. Cell debris was removed by centrifugation. The supernate containing the antigen was blotted in 2-mm wide lanes on to the nitrocellulose membrane by incubation for 2 h at 40°C. At the end of the incubation, the lanes of the blotting apparatus were rinsed with phosphate-buffered saline (PBS) to remove excess antigen. Blotted strips were rinsed with PBS, blocked with skimmed milk 3%, rinsed again and allowed to dry. The non-enzymic detection reagent consists of a monoclonal anti-human IgM antibody conjugated to a colloidal suspension of palanyl red [8, 9]. Briefly, the monoclonal antibody (MAb) was coated with a washed suspension of palanyl red in 10 mM phosphate buffer containing 2.7 mM NaCl. The concentration of the dye suspension was adjusted such that a 1 in 500 dilution gave a spectrophotometric A_{520} reading of 1. The conjugate was subsequently blocked with bovine serum albumin 30% in 5 mM NaCl. After blocking, the conjugate was suspended in 32.3 mM phosphate buffer containing 125 mM NaCl, trehalose 6% and 1.67 mM bovine serum albumin. Finally, the detection reagent was lyophilised to increase stability.

The dipstick assay was performed by incubating a wetted dipstick in a mixture of serum 5 µl and detection reagent 250 µl for 3 h at room temperature. At the end of the incubation, the dipsticks were thoroughly rinsed with water and dried. The staining intensity of the antigen band was then graded by comparison with a coloured reference strip. The test

was scored negative when no staining was observed, 1+ when weak staining was observed and 2+, 3+ or 4+ when moderate to strong staining was observed.

Results

S. typhi was isolated from bone marrow in 53 (86.9%) of 61 patients admitted to the Dr Kariadi Hospital with suspected typhoid fever. Of these 53 patients, 43 had a positive blood culture (81.1%). Also, one patient with a negative bone marrow culture had a positive blood culture. *S. paratyphi A* was isolated from bone marrow in another five patients. *S. typhi* was isolated in 37 (56.1%) of the 66 patients admitted to one of the district hospitals and in whom only blood culture was performed. *S. paratyphi A* was isolated in another three of these patients and *Staphylococcus aureus* and *Enterobacter* in one each.

The dipstick assay gave a positive result in 38 (70.4%) of 54 *S. typhi* culture-positive patients for whom blood and bone marrow culture was performed, and in 32 (86.5%) of 37 *S. typhi* culture-positive patients for whom only blood culture was performed (Table 1). The dipstick assay was positive in 37 (69.8%) of 53 patients from whom *S. typhi* isolated from bone marrow. In total, 70 (76.9%) of 91 patients with a *S. typhi* positive culture tested positive in the dipstick assay. The dipstick also gave a positive result in one (12.5%) of the patients with a positive culture for *S. paratyphi A*, and in four patients with a negative culture. Thirty-two of 36 patients with a negative culture for *S. typhi* were negative with the dipstick, indicating a high (88.9%) specificity of the assay. The high specificity was also confirmed by testing 80 patients with febrile illnesses other than typhoid fever. Of these, only three patients (3.8%), all with dengue haemorrhagic fever, gave a positive dipstick test.

The dipstick assay gave a moderate (2+) to strong (4+) staining intensity for 52 (74.3%) of the dipstick-positive patients with a *S. typhi*-positive culture. A weak (1+) staining intensity was obtained for the remaining patients (25.7%) with culture-confirmed typhoid fever and for the dipstick-positive patient with paratyphoid fever. The staining intensity of the three sera from the culture negative patients and the three dipstick-positive sera from the dengue haemorrhagic fever patients ranged from 2+ to 4+.

Among the culture-confirmed typhoid fever patients, six (8.5%) of 70 patients with a positive dipstick and two (10%) of 21 patients with a negative dipstick assay had complications.

The mean duration of fever before admission of the typhoid fever patients was 10.5 SD 4.9 days (range 5–35 days). Most culture-confirmed typhoid fever patients (65; 71.4%) were admitted during the second week of

Table 1. Results of the dipstick assay according to the results of culture in suspected typhoid fever patients and in control patients with other febrile illnesses

Results of culture	Results of dipstick assay					
	Number positive/ total (%)	Negative	1+	2+	3+	4+
Typhoid fever patients admitted to Dr Kariadi Hospital (n = 61)						
<i>S. typhi</i> positive*	38/54 (70.4)	16	13	18	7	0
<i>S. typhi</i> negative*†	1/7 (14.3)	6	1	0	0	0
Typhoid fever patients admitted to three district hospitals (n = 66)						
<i>S. typhi</i> positive‡	32/37 (86.5)	5	5	20	4	3
<i>S. typhi</i> negative‡§	3/29 (10.3)	26	0	1	1	1
All typhoid fever patients (n = 127)						
<i>S. typhi</i> positive	70/91 (76.9)	21	18	38	11	3
<i>S. typhi</i> negative	4/36 (11.1)	32	1	1	1	1
Control patients with other febrile illnesses (n = 80)	3/80 (3.8)	77	1	2	0	0

*Of 54 patients, *S. typhi* was isolated from blood and bone marrow in 43, from bone marrow alone in 10 and from blood alone in one patient.

†Including five *S. paratyphi A*-positive patients of whom one was dipstick-positive at a 1+ staining intensity.

‡In blood culture.

§Including three *S. paratyphi A*-positive patients.

||Including eight *S. paratyphi A*-positive patients of whom one was dipstick-positive at a 1+ staining intensity.

illness, 13 (14.3%) during the first week and another 13 (14.3%) after the second week of illness. A positive result in the dipstick assay was obtained in 84.6% of the culture-confirmed typhoid fever patients admitted during the first week of illness, in 75.4% of those admitted during the second week, and in 69.3% admitted during the third week. These results were not significantly different (χ^2 0.863, df = 2, p = 0.65).

Antibiotics had been used by 62.6% of the 91 blood or bone marrow culture-confirmed typhoid fever patients. The dipstick assay gave a positive result in 48 (84.2%) of 57 patients who had used antibiotics and in 22 (64.7%) of 34 patients who had not used antibiotics. The difference approached but did not reach statistical significance (χ^2 3.531, df = 1, p = 0.06).

Discussion

The sensitivity of the dipstick assay applied to a single serum sample collected at the time of admission to hospital was 69.8% when compared with bone marrow culture and 86.5% when compared with blood culture. Blood culture is considered the standard test to confirm the diagnosis of typhoid fever. The sensitivity of blood culture is highest (50–70%) during the first week of illness, drops rapidly beyond the first week and is reduced by antibiotic treatment [2, 7, 10]. A previous study in patients with typhoid fever found that the sensitivity of bone marrow culture, unlike blood culture, was not reduced by up to 5 days of prior treatment with chloramphenicol [4]. In the present study, most of the patients were admitted to hospital in the second week of illness and most patients had used antibiotics

before admission to the hospital. Compared with bone marrow culture, the sensitivity of blood culture was 81.1% confirming the observations of a previous study, which found that 19.3% of the patients with typhoid fever were missed if no bone marrow culture was performed [4]. However, bone marrow cultures are not usually performed in most clinical settings despite a relatively simple technique for collection of the sample.

Salmonella-specific IgM antibodies appear only after the first few days of disease and this may be influenced by antibiotic treatment. However, no difference in detection rate for culture-confirmed typhoid fever patients was observed between patients admitted during the first, second or third week of illness. Unexpectedly, the detection rate (84.2%) of the dipstick for culture-confirmed cases who reported the use of antibiotics prior to hospital admission was higher than the detection rate (64.7%) for patients who had not used antibiotics. Possibly, patients who used antibiotics had been infected with higher doses of *S. typhi*, making them more ill and hence more inclined to seek treatment.

A positive result in the dipstick test was obtained for four (11.1%) of 36 patients with clinically suspected typhoid fever for whom culture was negative. The specificity of the dipstick assay was thus calculated to be 88.9%. As culture is not always positive in cases of suspected typhoid fever, these four patients could have been true typhoid fever patients. Furthermore, cross-reactivity was observed in one patient with *S. paratyphi A* infection and in three patients with dengue haemorrhagic fever. Clinically, paratyphoid fever is similar to typhoid fever and treatment for this type of enteric

fever is the same. The cross-reactivity may be explained by the fact that *S. typhi* and *S. paratyphi* share common antigens and a relatively crude antigen was used in the dipstick assay. The three patients with dengue haemorrhagic fever were diagnosed clinically according to the WHO criteria [11]. These could still have been cases of typhoid fever who were at an early stage of the disease, because at this stage typhoid fever clinically mimics other febrile illnesses. Another possibility is that these patients had recently suffered from typhoid fever and then acquired dengue haemorrhagic fever.

The dipstick assay applied to a single serum sample gives a quick result (3 h). Testing of paired sera could increase the sensitivity of the assay and allow demonstration of seroconversion, providing stronger evidence of disease; however, the results would not be known for several days.

A diagnostic test may have a different sensitivity and specificity in different parts of the clinical spectrum of the disease. In this series, only eight patients with clinical suspicion of typhoid fever had complications. No difference was found in the positivity rate of the dipstick assay between culture-confirmed typhoid fever patients with and without complications.

In addition to sensitivity and specificity, the positive predictive value is an important characteristic of a diagnostic assay. The positive predictive value of the dipstick test was 94.6% at a prevalence of culture-confirmed patients among the suspected typhoid fever patients of 72%. Samples from suspected typhoid fever patients in four hospitals were tested, with an estimated prevalence of typhoid fever among patients with infectious diseases of *c.* 20%. The dipstick assay will be more useful for clinicians if applied to typhoid fever patients in a setting with a higher prevalence of the disease.

A number of serological tests to detect *S. typhi* antigen or antibodies have been developed; however, none of these tests is sufficiently sensitive, specific or rapid enough for clinical use [12, 13]. Serological tests like IgM ELISA and IgM capture ELISA can detect IgM antibodies in 62–81.8% of typhoid fever cases, but these tests are not practical and need a longer time than the dipstick assay [14, 15]. Dot-EIA [16] and other immunoassays [17, 18] showed good sensitivity and specificity for typhoid fever, but their relative complexity and costs limits their use to sophisticated laboratories. The validity of the classical Widal agglutination assay is influenced by a number of epidemiological and clinical factors, the quality of the antigen preparation and the way the assay is performed and interpreted [19–26]. Studies on the slide or tube Widal test in Indonesia have shown a moderate to high specificity but a low sensitivity [6, 23, 27]. Moreover, the Widal test is more complex and laborious than the dipstick

assay. As the dipstick assay uses stabilised components and does not require specific equipment for its performance, it can be used in settings without sophisticated laboratory facilities, even in rural areas without a refrigerator.

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