RAPID SEROLOGIC DIAGNOSIS OF PEDIATRIC TYPHOID FEVER IN AN ENDEMIC AREA: A PROSPECTIVE COMPARATIVE EVALUATION OF TWO DOT-ENZYME IMMUNOASSAYS AND THE WIDAL TEST

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Abstract. We evaluated the diagnostic sensitivity and specificity of two dot-enzyme-linked immunoassays (Typhidot® and Typhidot-M®; Malaysian Biodiagnostic Research SDN BHD, Kuala Lumpur, Malaysia), assessing IgG and IgM antibodies against the outer membrane protein (OMP) of Salmonella typhi, and the Widal test in comparison with blood culture in a consecutive group of children with suspected typhoid fever. Of 97 children with suspected typhoid fever, the disease was confirmed bacteriologically in 46 (47%), whereas 25 (26%) were considered to have typhoid fever on clinical grounds. An alternative diagnosis was made in 26 (27%). The Typhidot® and Typhidot-M® were superior to the Widal test in their diagnostic sensitivity and specificity, although values (sensitivity = 85–94% and specificity = 77–89%) were significantly lower than in other regional reports. The lower specificity of the Typhidot® in our series may represent regional differences in the genomic structure and plasticity of the OMP of S. typhi and merits further evaluation of these tests in diverse geographic locations.

Typhoid fever is widely recognized as a major public health problem in developing countries. It is estimated that there are more than 13 million cases occurring annually in Asia alone, of which a large proportion occur during childhood. In the wake of emerging multidrug-resistant strains of bacteria causing typhoid fever, the disorder is known to be associated with significant morbidity and mortality. It is also recognized that a delay in diagnosis and institution of appropriate therapy may significantly increase the risk of adverse outcome and mortality. Although the isolation of Salmonella typhi on blood culture remains the gold standard for diagnosing typhoid fever, this may be problematic in endemic areas where adequate microbiologic facilities are limited. The widespread availability and use of antibiotics in the community makes it frequently difficult to isolate the organism on blood cultures and alternative methods such as bone marrow cultures may be required. However, the latter are invasive and difficult to obtain routinely in pediatric patients.

Despite improved methods of bacteriologic isolation, there is a real need for rapid serologic diagnostic tests for typhoid fever. The Widal test has been used for almost 100 years old, is widely available in developing countries, and is still regarded as a useful test in endemic areas. There is, however, considerable interest in newer methods of diagnosis of typhoid fever such as latex agglutination, coagglutination, and the polymerase chain reaction. The dot-enzyme immunoassay (EIA) is a relatively newer serologic test based upon the presence of specific IgG and IgM antibodies to a specific 50-kD outer membrane protein (OMP) antigen on S. typhi strains and has been commercially marketed as a dot-EIA (Typhidot®; Malaysian Biodiagnostic Research SDN BHD, Kuala Lumpur, Malaysia). The test incorporates nitrocellulose strips impregnated with the OMP antigen and separately identifies IgM and IgG antibodies. Although the test has shown promising results in preliminary studies from Malaysia and The Philippines, the interpretation of the IgG response in highly endemic areas remains problematic. There is concern that in such endemic populations pre-existing IgG antibodies to S. typhi may increase rapidly following reinfection and potentially mask a concomitant IgM response. A recent, commercially available, enzyme-linked immunoassay (Typhidot-M®; Malaysian Biodiagnostic Research SDN BHD) is reported to circumvent these blocking antibodies by inactivating IgG antibodies, followed by an immunoassay targeting specific IgM. Preliminary data using the Typhidot® and Typhidot-M® in combination have shown sensitivity and specificity of 95% and 86%, respectively. Although the tests have shown promising results in trials from Southeast Asia, given the genetic diversity and plasticity of S. typhi strains, it is unknown if the test would be of comparable sensitivity in other regions.

We prospectively evaluated the efficacy of the two dot-EIA tests (Typhidot® and Typhidot-M®) in comparison with the Widal test in a consecutive group of children with suspected typhoid fever in Karachi, Pakistan.

MATERIALS AND METHODS

The study was performed at the Aga Khan University Medical Center, a 450-bed teaching hospital in Karachi. All children presenting to the ambulatory care services with suspected typhoid fever were evaluated according to a set protocol. Detailed clinical evaluation and profile were obtained and a typhoid fever morbidity score was calculated as described previously. In all cases a complete blood count and malarial blood film were obtained and liver function tests were conducted. Five milliliters of blood were inoculated into 2 blood culture bottles containing brain heart infusion broth and thioglycollate broth, respectively. In cases who had received antibiotic therapy for ≥ 72 hr, a bone marrow culture was also obtained as described previously. The cultures were examined thereafter for bacterial growth at different stages, subcultured, and positive colonies were biochemically identified with analytical profile index 20E strips (Analytab Products, Plainview, NY). The Widal test was performed by preparing serial tube dilutions of patient sera in 0.85% saline and mixing standard preparations of Salmonella O and H antigens (Wellcome Diagnostic, Dartford, United Kingdom), followed by incubation at 50°C for 24 hr. H and O agglutination were characterized by large flocculent or smaller granular aggregates, respectively. Results were
expressed as the inverse of the highest dilution expressing agglutination.

The dot-EIA tests were performed using standard commercial kits (Typhidot® and Typhidot-M® (generous donations from Malaysian Biodiagnostics Research, Kuala Lumpur, Malaysia) containing nitrocellulose strips dotted with 0.3 \( \mu \text{g} \) of the 50-kD OMP. The Typhidot® strips were probed with 1:100 dilution serum, washed with phosphate-buffered saline, and 1 hr later the antigen-antibody complexes were visualized by addition of horseradish peroxidase–conjugated antiserum to human IgG and IgM and 4-chloro-1-naphthol. Positive results were read in comparison with control sera and represented a titer \( > 1:100 \). A positive IgG or IgM result, either alone or in combination, was regarded as a positive test result in our patients. The Typhidot-M® differed in the initial steps: the addition of an IgG-inactivation complex and subsequent removal of the bound-IgG with anti-human IgG labeled with horseradish peroxidase. The subsequent antibody binding and color change identified primarily IgM antibodies against the OMP.

The protocol of the study was approved by the Human Subjects Protection Committee--Aga Khan University Medical Center, and informed consent was obtained from parents/guardians of all children prior to sampling. In all cases the sensitivity and specificity of the tests were calculated along with corresponding positive and negative predictive values. These parameters were calculated for the entire group of typhoid fever patients (including both culture-proven and clinical typhoid cases) as well as the non-typhoidal controls, as well as the subcohort of culture-proven typhoid fever cases alone. Data were comparatively evaluated using analysis of variance and the chi-square test, as appropriate.

### RESULTS

A total of 97 children presented to the ambulatory services over a 6-month period with suspected typhoid fever. The diagnosis was confirmed in 46 (47%) of 97 by the isolation of \textit{S. typhi} on blood and/or bone marrow cultures. In 25 cases (26%), although successive cultures were negative, a clinical diagnosis of typhoid fever was made and these patients were treated as such with either first or second-line antibiotics (oral amoxicillin or intravenous ceftriaxone). In 26 cases (27%), an alternative diagnosis was made after 48–72 hr, and treatment with the antibiotics was stopped. These latter cases constituted negative controls and consisted of 8 cases with viral respiratory infection, 4 with pneumonia, 4 with urinary tract infections, 3 with malaria, 3 with bacterial diarrhea, 3 with rheumatoid arthritis, and 1 with viral meningitis. All cases were followed up for 8–12 weeks after presentation.

Table 1 compares the admission characteristics of the 3 groups of patients. Children with typhoid fever tended to be older, and had significantly longer duration of illness at presentation (12.8 ± 11.0 versus 9.1 ± 4.0 days [mean ± SD]; \( P < 0.05 \)). They also had higher levels of alanine aminotransferase at admission (57.5 ± 96.4 versus 29.6 ± 31.4 IU/L; \( P < 0.05 \)) and were significantly more ill with higher values for the typhoid fever morbidity score (3.9 ± 2.2 versus 3.0 ± 1.9; \( P < 0.05 \)).

Table 2 details the sensitivity, specificity, and positive and negative predictive values for the Widal, Typhidot®, Typhidot-M®, and blood/bone marrow cultures for this cohort. The combination of cultures and Typhidot-M® offered the highest diagnostic sensitivity and specificity for diagnosing ty-
phoid fever. When only the blood/bone marrow culture-proven cases (n = 46) were analyzed, both the Typhidot® and Typhidot-M® were significantly superior to the Widal test in terms of the diagnostic predictive value (Table 3).

**DISCUSSION**

In contrast to findings from other parts of Asia,20–22 our data support the contention that the Widal test has poor diagnostic value in children with typhoid fever.23,24 Most of the children presented in the second week of their illness and we used a cut-off titer of 1:80. The use of a higher cut-off titer would have further reduced the sensitivity of the test. Although this has been questioned,25 antibiotic therapy has been shown to alter the antibody response to *S. typhi* infection,26 and given that a large number of the patients had previously received antibiotics, this factor may have altered the antibody titers against O antigens. The Typhidot® was significantly more sensitive than the Widal test, although the sensitivity and specificity were lower than those reported from Malaysia and The Philippines.27–29 Our findings of sensitivity and specificity were also lower than values > 90% reported recently by Karamat and others.27 from northern Pakistan. These differences may be due to several factors including the genomic diversity among *S. typhi* isolates in the region28 and differences in antigenic epitopes. Other factors responsible for reported differences in areas of high endemicity are various stages of the illness and the rate of increase of IgG antibodies to the OMPs, which may interfere with identification of concomitant IgM antibodies. Most of our patients presented in the second week of their illness, whereas information on duration of illness is lacking in other studies.10,11,28

The relative low sensitivity of the blood culture in diagnosing typhoid fever is understandable in the wake of widespread antibiotic use in Pakistan29 and the difficulties of obtaining large enough blood volumes for cultures from children. Although bone marrow cultures significantly increase the yield from cultures,4,30 they are invasive and difficult to obtain. It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among *S. typhi*, they remain an essential investigation. In many circumstances, especially among partially treated cases presenting to health facilities, combining cultures with a rapid serologic test may reduce the diagnostic difficulty in typhoid fever. Our data indicate that combining the blood/bone marrow cultures with a Typhidot-M® will significantly improve the diagnostic yield of these investigations among children who have previously received antibiotics. We do not believe that our data support the use of either the Widal or Typhidot® tests as a substitute for cultures in typhoid fever.

The Typhidot® offers an additional advantage among second-line serologic diagnostic tests for typhoid fever in that the test strips do not require an ELISA reader for evaluation. Also, only minimal operator training is required. Nevertheless, the 3–4-fold higher cost of the test in comparison with the Widal test, as well as cold-storage requirements for test reagents, are additional impediments in using this test in developing country. Although combining the Typhidot® and Typhidot-M® tests may improve sensitivity, this is an expensive proposition. Given the recent call for an essential diagnostics program in developing countries,31 it is important that the Typhidot® and Typhidot-M® tests be evaluated on a larger scale in different parts of the world with epidemiologically diverse strains of *S. typhi*.

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