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RESEARCH

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Use of onsite typhoid IgG/IgM combo test as rapid diagnostic test for typhoid fever

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Abstract

Background The incidence of typhoid fever is increasing in Egypt. The Widal test is the evaluation most widely used in Egypt for diagnosis, but it has many drawbacks; therefore, new diagnostic tools are needed. Our aim was to evaluate the diagnostic accuracy of the onsite typhoid IgG/IgM combo rapid test in diagnosing typhoid fever.

Method Blood specimens were collected from 90 patients (of all ages) who presented with fever of more than 4 days' duration. The OnSite Combo test and the Widal test were performed for all patients.

Results The OnSite Combo test results were positive in approximately 24% of all patients; the Widal test results were positive in 18.9%; and typhoid was diagnosed through blood culture in 32.2%. The OnSite Combo test had 72.4% sensitivity, 98.4% specificity, a positive predictive value of 95.5%, and a negative predictive value of 88.2%. In contrast, the Widal test had 51.7% sensitivity, 69.7% specificity, a positive predictive value of 88.2%, and a negative predictive value of 80.8%.

Conclusions The onsite combo test was more efficacious than the Widal test in diagnosing typhoid fever.

Keywords Typhoid, Onsite typhoid IgG/IgM combo rapid test, Widal test, Blood culture

1 Background

Many countries experience typhoid fever, which is typically found in less developed regions due to poor sanitation. Asia, Latin America, Africa, the Caribbean, and Oceania are among the regions where typhoid fever is prevalent; 80% of occurrences occur in Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan, and Vietnam. About 21.6 million individuals worldwide are affected by typhoid fever (incidence of 3.6 per 1000 population), and it claims 200,000 lives annually [1].

Typhoid fever can present as a mild illness with low-grade fever, headache, fatigue, malaise, loss of appetite, cough, constipation, and skin rash or rose spots to a serious illness with serious complications like intestinal perforations, gastrointestinal hemorrhages, encephalitis, and cranial neuritis. Despite the clinical significance of typhoid fever, laboratory diagnosis relies heavily on nonspecific clinical signs and the results of the Widal test, which has low specificity and low positive predictive value, in countries with inadequate resources (PPV) [2].

Not all diagnostic techniques work as well as others. The most sensitive method is bone marrow bacterial culture (sensitivity > 80%). Bone marrow aspiration and culture are a costly and intrusive procedure, hence they are not frequently employed in clinical settings. Consequently, blood culture remains the practical gold standard for diagnosing typhoid fever, despite being less sensitive [3].

When used as a diagnostic tool, the Widal test has subpar sensitivity and specificity; up to 30% of cases of typhoid fever with culture-proven causation may have negative results, especially early in the infection and in patients who have previously received antibiotic therapy. [4].

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Therefore, a low-cost, highly accurate test is required to diagnose typhoid disease. We evaluated the onsite typhoid IgG/IgM combo rapid test CE (CTK Biotech, Poway, CA, USA diagnostic's accuracy in patients who presented with fever and other symptoms of typhoid fever and in whom the disease was diagnosed by blood culture, using blood culture values as references.

2 Methods

Blood samples were taken from patients who had fever for more than 4 days. Three laboratory tests were performed.

(1) The OnSite Combo Test is an immunoassay useful for the detection and discrimination of immunoglobulin G (IgG) and M (IgM) anti-Salmonella antibodies. (2) Widall test. (3) Blood culture.

Patients of all ages and genders were tested. Inclusion criteria were:

A- The presence of fever for ≥ 5 days.
B- Presence of fever and other clinical signs and symptoms of typhoid fever
C- Confirmation of typhoid fever by blood culture.
D- Immunocompromised patients were excluded

2.1 "Specimen collection"

Ninety patients who met the inclusion criteria provided informed consent to participate in the study and were asked to complete a brief questionnaire regarding clinical signs and symptoms, antimicrobial treatment, typhoid fever and vaccination history. Laboratory and blood samples were taken from the patients. The volume of blood collected by routine venipuncture was 10 mL in adults and 3 mL in children (ages 3–5). Blood samples were assayed for Salmonella enterica serovar Typhimurium according to standard laboratory blood culture protocols. Each 5 or 3 mL sample of fresh blood was inoculated into blood culture bottles containing 45 mL of broth each and incubated at 37 °C for 24 h. Specimens negative at 24 h were cultured for an additional 7 days. Those that were positive were plated on MacConkey agar, chocolate and blood agar plates and incubated at 37 °C for 24 h. Plates were examined for colonies of S. typhi serovar Typhimurium. To confirm the presence of microorganisms, standard biochemical tests and serological tests using slide agglutination with the following antisera were performed. Salmonella factor O, Salmonella H polyvalent phases 1 and 2 (a-z29), and Salmonella H monofactor d (because S. enterica serovar Typhimurium reacts positively with all of them). Blood samples were also used for parallel evaluation with the OnSite Combo Rapid Test performed according to the manufacturer's instructions.

2.2 Evaluation of diagnostic accuracy of the rapid test kits

OnSite Combo Test results were compared to blood culture values. Sensitivity, specificity, PPV and NPV were calculated using standard formulas. Sensitivity was calculated as the number of true positive (TP) cases/(number of TP cases + number of false-negative [FN] cases) × 100. Specificity was calculated as the number of true negative (TN) cases/(number of TN cases + number of false-positive [FP] cases) × 100. PPV was calculated as the number of TP cases/(TP + FP cases) × 100. NPV was calculated as TN cases/(TN cases + FN cases) × 100%.

2.3 Statistical analysis

The collected data were coded and then analyzed with SPSS version 25 (IBM Corporation, Armonk, NY, USA). Categorical variables were calculated as frequencies and percentages, and numerical variables were calculated as means and standard deviations. We used suitable statistical tests of significance: the independent-samples *t* test for two unrelated samples; the chi-square test or Fisher's exact test for categorical data; and Spearman's correlation analysis, whereby *r* values of 0–0.3 represented weak correlations, > 0.3 to 0.6 represented moderate correlations, and > 0.6 to 1 represented strong correlations. Statistical significance was indicated by p values of ≤ 0.05. We used simple graphs to illustrate some information. Receiver operating characteristic curves were used to predict the optimal cutoff value of ferritin in the prediction of significant fibrosis.

3 Results

Ninety patients who met the inclusion criteria provided informed consent to participate in the study and were asked to complete a brief questionnaire regarding clinical signs and symptoms, antimicrobial treatment, typhoid fever and vaccination history. Laboratory and blood samples were taken from the patients. The mean age of the patients was 22.28 ± 6.3 years (range, 3–35 years); 63.3% were male and 36.7% were female. All patients had fever; among the 33.3% who had abdominal pain, typhoid was diagnosed in 30%. Among the 22.2% of patients who had headache, typhoid was diagnosed in 25%. Among the 27.8% who had constipation, typhoid was diagnosed in 36%. Among the 14.4% who had diarrhea, typhoid was diagnosed in 30.8%. Among the 4.4% who had cough, typhoid was diagnosed in 25%. Among

Table 1 Clinical data of studied patients:

Variable	Number $T=90$	Percentage (100%)	Confirmed to be diseased
Fever	90	100	29 (32.2%)
Abdominal pain	30	33.3	9 (30%)
Headache	20	22.2	5 (25%)
Constipation	25	27.8	9 (36%)
Diarrhea	13	14.4	4 (30.8%)
Cough	4	4.4	1 (25%)
Coated tongue	3	3.3	2 (66.7%)
Rash	1	1.1	0 (0%)
Hepatosplenomegally	5	5.6	2 (40%)

Table 2 Results of Combo test compared to diagnostic blood culture

	Combo test Positive	Blood culture Negative	Total
Positive	True positive 21	False positive 1	22
Negative	False negative8	True negative60	68
Total	29	61	90

Table 3 Results of Widal test compared to diagnostic blood culture

Widal test	Blood culture		Total
	Positive	Negative	
Positive	True positive 15	False positive 2	17
Negative	False negative 14	True negative 59	73
Total	29	61	90

Table 4 Results of different diagnostic tests

Test	Positive *N* (%)	Negative *N* (%)	Total
Combo	22(24.4%)	68 (75.6%)	90 (100%)
Widal	17 (18.9%)	73 (81.1%)	90 (100%)
Blood culture	29 (32.2%)	61 (67.8%)	90

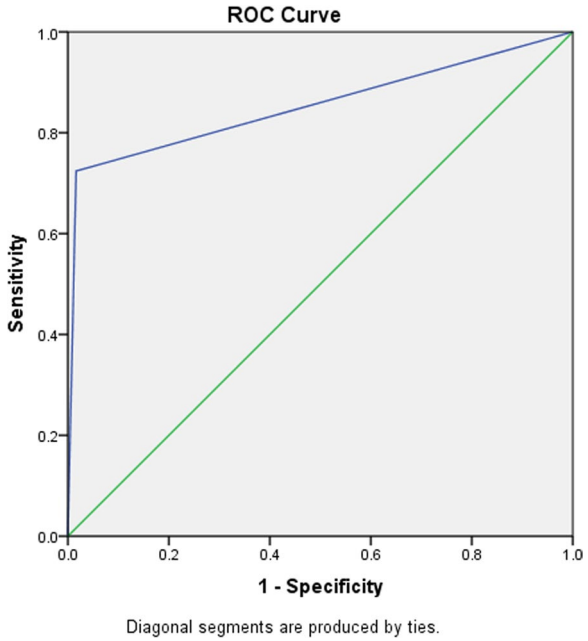

Fig. 1 Rock curve of combo test using blood culture as a reference

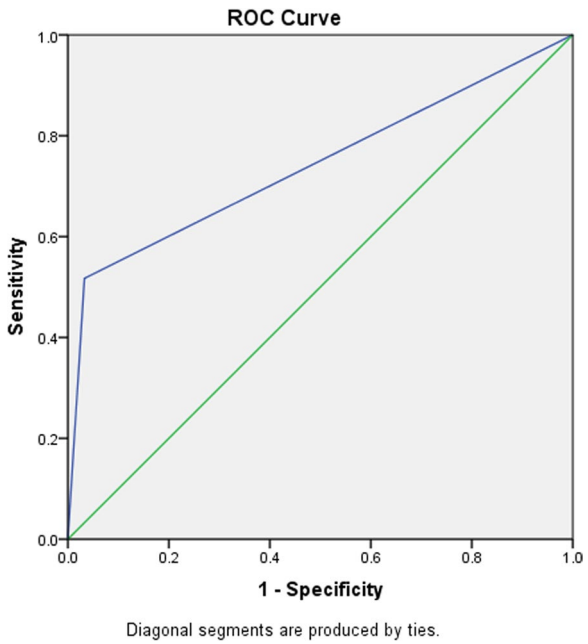

Fig. 2 Rock curve of widal test using blood culture as a reference

the 3.3% who had coated tongue, typhoid was diagnosed in 66.6%. Typhoid was not diagnosed in any of the 1.1% who had a rash. Among the 5.6% who had hepatosplenomegaly, typhoid was diagnosed in 40% (Tables 1, 2, 3 and 4; Figs. 1 and 2).

The OnSite Combo test was 72.4% sensitive (21/29) and 98.4% specific (60/61); the PPV was 95.5% (21/22), and the NPV was 88.2% (60/68). In contrast, the Widal test was 51.7% sensitive (15/29) and 69.7% specific (59/61), had a PPV of 88.2% (15/17) and a NPV of 80.8% (59/73). Thus the OnSite Combo test had higher sensitivity, specificity, PPV, and NPV than the Widal test.

The OnSite Combo test results were positive in 24% of the patients; the Widal test results were positive in 18.9%;

and 32.2% of typhoid cases were diagnosed through blood culture.

4 Discussion

The main aim of this study was to evaluate the accuracy of the OnSite Combo test in diagnosing typhoid fever. The OnSite Combo test was 72.4% sensitive (21/29) and 98.4% specific (60/61); the PPV was 95.5% (21/22), and the NPV was 88.2% (60/68). In contrast, the Widal test was 51.7% sensitive (15/29) and 69.7% specific (59/61), had a PPV of 88.2% (15/17) and a NPV of 80.8% (59/73) so the OnSite Combo test was more efficacious than the Widal test, according to its greater sensitivity, specificity, PPV, and NPV. Our statistics were higher than those shown by Rapeephan et al. [4], who evaluated the diagnostic accuracy of three rapid diagnostic tests for typhoid fever in febrile hospitalized patients in Bangladesh; in their study, the sensitivity and specificity of the OnSite Combo test were 59% and 74%, respectively.

Our results are also consistent with those of Tarupiwa et al. [5], who found that the OnSite Combo test was useful for the rapid diagnosis of *S. enterica* infection: it was 100% sensitive and 94.35% specific and had a PPV of 63.16% and a NPV of 100%.

According to CTK biotec 2007 [6], a total of 234 samples from susceptible subjects were evaluated by the onsite typhoid IgG/IgM combo rapid test and with a commercial *Salmonella enterica* serovar Typhi IgM EIA as a standard, sensitivity and specificity were 91.1% and 99.0% respectively which was comparable with ours.

Jason et al. [7] reported that widal test suffers from significant limitations in its sensitivity and specificity, as well as reliability. Like most serologic tests, a false-negative Widal test may occur early in the course of illness, and a false-positive Widal test may result from past infection or from previous exposure to cross-reactive antigens or vaccination. There are no universal standards that define the cutoff dilution of agglutinating antibodies to indicate a positive Widal test. The very low specificity of the assay (50–70%) and the inability to discern active from previous infection or vaccination means that the assay should rarely, if ever, be used, which is consistent with results of our study that revealed that the Widal test was 51.7% sensitive (15/29) and 69.7% specific (59/61), had a PPV of 88.2% (15/17) and a NPV of 80.8% (59/73).

According to who, 2018 Symptoms of typhoid include prolonged fever, fatigue, headache, nausea, abdominal pain, and constipation which are consistent with this study in which fever was main presentation followed by abdominal pain headache and constipation.

In study done by Habte et al. [8] fever, headache, abdominal pain and constipation was predominant clinical presentation of culture confirmed typhoid cases which goes with our research.

Hosoglu et al. [9] and Vollaard et al. [10] showed that the manifestation of typhoid fever that is most commonly reported is fever followed by diarrhea, abdominal pain, headache, splenomegaly, constipation, and hepatomegaly. Those results, too, are consistent with our clinical data.

Study done by Neopan [11], diagnostic criterion was proposed and clinical features with diagnostic accuracy more than 50% were taken into consideration for typhoid fever major criteria included fever with diagnostic accuracy of 64%, headache with accuracy of 75.5% and relative bradycardia with an accuracy of 66%. Minor criteria included vomiting, diarrhea, Splenomegaly, chills and abdominal pain /discomfort with diagnostic accuracy of 57%, 55%, 55%, 53% and 51% respectively. Finally after combination of various major and minor criteria a final diagnostic criterion was proposed having an accuracy of 66% and including both major and minor clinical symptom and sign which goes with our findings.

Our study goes with Ousenu et al. [12] in which main cinical presentation was fever followed by headache and abdominal pain but goes against as regard diagnostic accuracy of widal test which was 94% much higher than ours.

This study goes in accordance with study done by Bernard et al. [13] who establishes that, Widal test has a moderate diagnostic accuracy with average percentage sensitivity (52.9%), specificity (54%), positive predictive value (PPV) (56.8%) as well as negative predictive value (NPV) (55.6%).

Razia et al. [14] reported in study enrolled on 265 patients that sensitivity and specificity of the combo test were reported to be 96.9% and 88.7% and low sensitivity of widal test 36% that supports the higher diagnostic accuracy of combo test in comparison with widal test which goes with our results..

In contrast study done by Sapcota et al. [15] Combo test have low sensitivity and specificity which goes against our results.

In study done by Rapeephan et al. [16] fever, headache and abdominal pain were main symptoms which were compatible with our clinical data and as regard diagnostic accuracy of combo test sensitivity of combo test was 58.8% and specificity was 73.7% which was lower than that settled in our research.

5 Conclusion

Widal test has many draw backs as regard low sensitivity, specificity, false-positive results from previous exposures to infection and so we investigated diagnostic accuracy of OnSite Combo test and results revealed

The OnSite Combo test was 72.4% sensitive (21/29) and 98.4% specific (60/61); the PPV was 95.5% (21/22), and the NPV was 88.2% (60/68). In contrast, the Widal test was 51.7% sensitive (15/29) and 69.7% specific (59/61), had a PPV of 88.2% (15/17) and a NPV of 80.8% (59/73). Thus the OnSite Combo test had higher sensitivity, specificity, PPV, and NPV than did the Widal test and so we concluded that The OnSite Combo test, a simple, rapid, economic tool more efficacious than the Widal test in diagnosing typhoid fever.

Abbreviations

FN	False negative
FP	False positive
NPV	Negative predictive value
PPV	Positive predictive value
SPSS	Statistical package for social science
TP	True positive
TN	True negative

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Not applicable

Author contributions
AA analyzed and interpreted the patient data. BE supervised data collection and analysis. RR helped in data analysis. MM collected the data. All authors have read and approved the manuscript.c

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Not applicable.

Declarations

Ethics approval and consent to participate
This study was approved by Ethics Committee of Beni-Suef University, Faculty of medicine and the ethics code was FWA00015574. Also, written consent was obtained from the care givers of the participating patients.

Consent for publication
Not applicable.

Competing interest
The authors declare that they have no competing interest.

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