Research Article (Open access)

Comparative Evaluation of ENTEROSCREEN-WB™ and Widal test in Suspected Cases of Enteric Fever

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ABSTRACT- Enteric fever is a major public health problem in developing countries like India. An early and accurate diagnosis is necessary for a prompt and effective treatment. We have evaluated the diagnostic accuracy of ENTEROSCREEN-WB™ as compared to Widal test in rapid and early diagnosis of enteric fever. A total of 145 patients serum samples were tested by Rapid ENTEROSCREEN-WB™ and Widal test including clinically suspected cases of enteric fever of all age groups. Vaccinated individuals, patients on antibiotic therapy, patients who have other associated conditions, patients suffering from fever due to non-enteric etiology & non consent patients were excluded. The overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ENTEROSCREEN-WB™ considering Widal test as gold standard were 50% and 96%, 66.66% and 92.30% respectively. ENTEROSCREEN-WB™ was found to be significantly more specific. Although the Rapid ENTER-OSCREEN-WB™ tests are meant to diagnose of *S. typhi*. Ten patients who were ENTEROSCREEN-WB™ positive for *S. typhi* were also positive by Widal test.

Key words- Enteric fever, Rapid ENTEROSCREEN-WB™, Non-enteric etiology, *S. typhi*, Widal test

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INTRODUCTION

Typhoid fever is an acute, generalized infection of the reticulo-endothelial system, intestinal lymphoid tissues and gallbladder caused by *Salmonella typhi*[1]**.** The annual incidence of typhoid fever has been reported as more than 13 million cases in Asia[2]. Typhoid fever is endemic and one of the commonest infectious diseases prevalent in India[3]. India is the second most populous country of the world with majority inhabiting the rural areas with little access to modern diagnostic tools[4, 5]. The isolation of the organism from blood, bone marrow or stool is required to confirm the diagnosis, which is time consuming[3]. Blood culture is regarded as the gold standard for diagnosis & carry 70-75% diagnostic yield in the first week of illness[6]. In WIDAL test the agglutination titer will depend on the stage of disease.

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Agglutinins will usually appear by the end of the 1st week, so that blood taken earlier may give a negative result. The titer increases steadily until the third or the 4th week, after which it declines gradually[7].

ENTEROSCREEN-WB™ is a rapid dot-enzyme immune assay (EIA), which detects IgG and IgM antibodies to a specific outer membrane protein (OMP) antigen of Salmonella enteric serotype Typhi. Typhi dot becomes positive as early as in the first week of fever; the results can be visually interpreted and is available within one hour[8].

MATERIALS AND METHOD

SPECIMENS

Blood for culture must be taken repeatedly. In enteric fevers and septicemias, blood cultures are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine cultures may be positive after the second week.

BACTERIOLOGIC METHODS FOR ISOLATION OF SALMONELLAE

Differential Medium Cultures- MacConkey's or Deoxycholate medium permits rapid detection of lactose non-fermenters. Bismuth sulfite medium permits rapid detection of salmonellae which form black colonies because of H_2S production. Many salmonellae produce H_2S.

Selective Medium Cultures-The specimen is plated on *salmonella-shigella* (SS) agar, Hektoen enteric agar, XLD, or Deoxycholate-Citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae.

Enrichment Cultures- The specimen (usually stool) also is put into selenite F or tetrathionate broth. After incubation for 1–2 days, this is plated on differential and selective media.

Final Identification- Suspected colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

SEROLOGIC METHODS

Tube Dilution Agglutination (Widal Test)- The Widal test, which detects agglutinating antibodies to lipopolysaccharide (LPS) (TO) and flagella (TH), was introduced over a century ago and is widely used for the serological diagnosis of typhoid fever. In the original format, the Widal test required acute and convalescent phase serum samples taken approximately 10 days apart. Most recently, the test has been adapted for use with a single, acute phase serum sample. This is a test for the measurement of H and O agglutinins for typhoid and paratyphoid bacilli in the patient's sera. Equal volumes of serial dilutions of the serum and O, H, AH, BH antigens were mixed in the test tubes and incubated in a water bath at 37^0C overnight.

Rapid Diagnostic Test (ENTEROSCREEN-WB™ device) ENTEROSCREEN-WB™ utilizes the principle of immunochromatography, a unique two-site immunoassay on a nitrocellulose membrane. ENTEROSCREEN-WB™ is a dual test device assembly comprising of an IgM detection test assembly and an IgG detection test assembly. The conjugate pad of the IgM test assembly consists of two components, the Anti-human IgM antibody conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold. Similarly the IgG test assembly consists of Anti-human IgG antibody conjugated to colloidal gold and rabbit globulin conjugated to colloidal gold.

RESULTS

During the study period 145 suspected cases of enteric fever attending IIMS&R were concurrently tested for a rapid test ENTEROSCREEN-WB™ and Widal test. 15 samples were positive by ENTEROSCREEN-WB™ and 20 were positive by Widal test including 10 samples which were positive by both Widal test and ENTEROSCREEN-WB™.

Table 1: Comparative evaluation of Widal test and ENTEROSCREEN-WB™

Name of Test	N (%)	ENTEROSCREEN-WB™ Positive	ENTEROSCREEN-WB™ Negative
Widal Test Positive	20 (13.79%)	10	10
Widal Test Negative	125 (86.20%)	05	120
Total	145	15 (10.34%)	130 (89.65%)

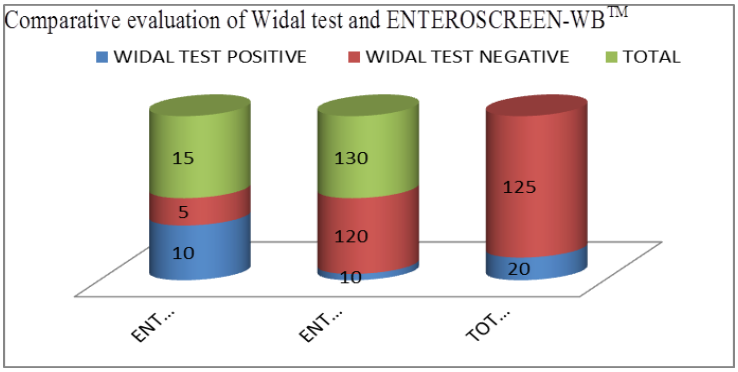

Fig. 1: Subdivided bar diagram showing comparative evaluation of Widal test and ENTEROSCREEN-WB™

Among the total 145 samples, 20 cases (13.79%) showed positive result by Widal test and 15 cases (10.34%) showed positive result by ENTEROSCREEN-WB™.

Table: 2: Comparision of positivity rate of Widal test and ENTEROSCREEN-WB™

Total Sample (n=145)	Positive	Negative
Widal Test	20 (13.79%)	125 (86.20%)
ENTEROSCREEN-WB™	15 (10.34%)	130 (89.65%)

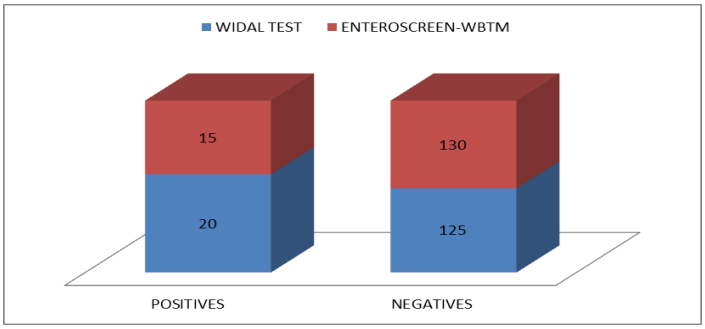

Fig. 2: Subdivided bar diagram showing comparision of positivity rate of Widal test and ENTEROSCREEN WB™

The distribution of *Salmonella* antibodies, 12 and 11 cases were positive for *Salmonella* 'O', 'H' antibody respectively. The patients were between the age of 0- >61 years. Females of 20-30 years and males of 1-10 years age group constituted 29.87% and 27.94% respectively. However

females had higher incidence rate of enteric fever as compared to males. Male female ratio was 0.88:1.

Table 3: Age and sex distribution of Widal test and ENTEROSCREEN-WB™

Age (yrs.)	Total no.	Sex distribu-tion		Positive widal test			Positive ENTEROSCREEN-WB™		
	N (%)	M	F	M	F	Total	M	F	Total
1-10	31(21.37)	19	12	2	2	4	2	2	4
11-20	25 (17.24)	7	18	1	4	5	3	2	5
21-30	39 (26.89)	16	23	3	6	9	2	2	4
31-40	20 (13.79)	11	9	0	1	1	0	1	1
41-50	9 (6.20)	4	5	0	0	0	0	0	0
51-60	10 (6.89)	6	4	0	1	1	0	1	1
> 60	11 (7.58)	5	6	0	0	0	0	0	0
	145	68	77	6	14	20	7	8	15

M= MALE, F= FEMALE,* FIGURE IN PARENTHESIS INDICATES PERCENTAGE

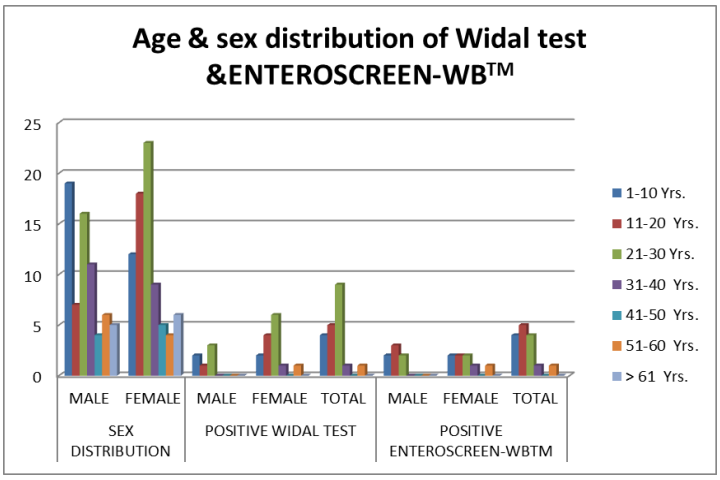

Fig. 3: Cluster column chart showing age and sex wise distribution of Widal test and ENTEROSCREEN-WB™

Following table shows Department wise distribution of blood samples revealed that Medicine department had the highest contribution followed by the Pediatrics & Obs & Gynae.(Table:6) (Figure: n).

Table 4: Department wise distribution of samples

Departments	No. Of samples
Medicine	91
Pediatrics	41
Obs & Gynae	13

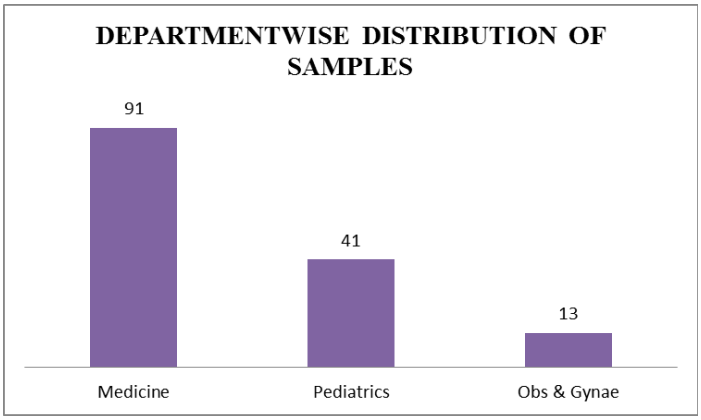

Fig. 4: Cluster column chart showing department wise distribution of samples

Fever was present in 129 (88.96%) cases followed by body-ache 122 (84.13%) and weakness 98 (67.58%) cases. p-value is significant in abdominal pain, diarrhea, headache, nausea/vomiting and sweating.

TABLE 5: CLINICAL FEATURES OF TOTAL CASES OF ENTERIC FEVER

Sensitivity	50%
Specificity	96%
PPV	66.66%
NPV	92.30%

Symptoms (n=145)	Positive	Negative	Chi-square value	p-value
Abdominal pain	25 (17.24%)	120	48.3	**0.001**
Bodyache	122 (84.13%)	23	1.83	0.97
Chills	17 (11.72%)	128	0.84	0.77
Diarrhea	59 (40.69%)	86	6.12	**0.01**
Fever	129 (88.96%)	16	0.12	0.72
Headache	50 (34.48%)	90	14.8	**0.001**
Malaise	81 (55.86%)	64	0.26	0.87
Nausea/vomiting	5 (3.44%)	140	13.1	**0.001**
Sweating	56 (38.62%)	89	11.6	**0.001**
Weakness	98 (67.59%)	47	2.58	0.108

p- Value (<0.05) = significant

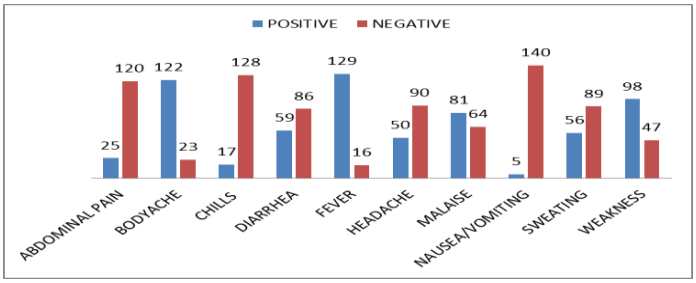

Fig. 5: Cluster column chart showing clinical features of total cases of enteric fever

Sensitivity(50%), specificity (96%), PPV (66.66%), NPV (92.30%) of ENTEROSCREEN-WB™ as gold standard Widal test.

True positive (a) =10, False positive (b) =5, False negative (c) =10, False positive (d) =120

Sensitivity- a/a+c X 100= (10/10+10 x 100) = 50%

Specificity- d/d+b X 100= (120/125 x 100) = 96%

PPV-Positive Predictive Value- a/a+b x 100= (10/15 x 100) = 66.66%

NPV-Negative Predictive Value- d/d+c X100 = 92.30%

Table 6: Sensitivity, Specificity, PPV and NPV of ENTEROSCREEN-WB™

Total Sample (n=145)	Widal test positive	Widal test negative	N (%)
ENTEROSCREEN-WB™ POSITIVE	(a) 10	(b) 5	15
ENTEROSCREEN-WB™ POSITIVE	(c) 10	(d) 120	130
Total			145

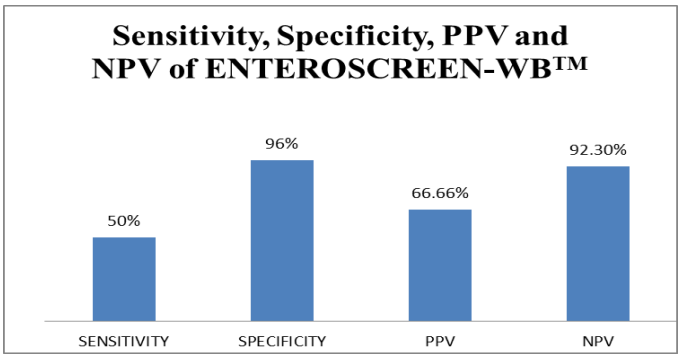

Fig. 6: Cluster column chart showing Sensitivity, Specificity, PPV and NPV of ENTEROSCREEN-WB™

DISCUSSION

Typhoid fever is a systemic illness with significant morbidity and mortality in developing countries. Poor sanitation, overcrowding, lack of medical facilities, and indiscriminate use of antibiotics lead to endemicity of typhoid fever and multi-resistant strains of *Salmonella typhi* in these countries [9,10].

In present study a total of 145 clinically suspected enteric fever cases were included. The present study includes all the age group. Out of 145 cases, 68 were male patients, 77 were female patients including 31 children and male and female ratio in the present study is 0.88:1. In the present study, 20 cases were positive by Widal test and 15 cases were positive by ENTEROSCREEN- WB™. The disease affected all ages, however most of the cases 31 (21.37%) of the study were in the age group of 1-10 years. This findings correlates with the observation made by[11] who found that children between 2-3 years of age are most susceptible age group (35.6%) [11]. Another study from Bangladesh done by [12] showed that 16.2% patients in the age group of 2-3 years are more susceptible to infection[12].

Almost similar studies done by 13 showed 44% children suffering from enteric fever were aged less than 5 years[13]. In the present study, among 145 clinically suspected typhoid cases 47% were male and 53% were female, which is in contrary with [14] also showed that infection rate is slightly higher in male, perhaps reflecting greater exposure of male to contaminated food and water outside the home.

In a study done by [15], out of 50 cases studied, 33 were positive by blood culture, 33 were positive by Widal test (it includes 26 blood culture positives) and 37 were positive by Typhi-dot[15]. In contrary to this finding, present study out of 145 cases studied, 20 were positive by Widal test and 15 were ENTEROSCREEN- WB™ positive. In another study from India by Nakhla and others showing contrary type of result that the sensitivity and specificity of ICT (IgM) was 80% and 71.4% respectively[16]. ICT has been studied in many countries and they found significantly higher sensitivity and specificity [17-19].

A contrary study carried out in the southern part of India reported typhi-dot of having a sensitivity of 100% and a specificity of 80% and was recommended for its utility in conjunction with Widal test for an early diagnosis of typhoid fever, compared to blood culture as a 'gold standard'[17].

Two studies were similar to present study, one from Pakistan [20] showed that only 7 (28%) out of 25 and one from Malyasia [21] showed that 18 (36%) out of 50 culture negative clinical typhoid fever cases were DOT EIA positive. This was probably due to the fact that they have used the clinical feature rather than rise in Widal titre for the diagnosis of typhoid fever cases which is very much variable and unreliable**.**

In the present study 15 (10.34%) cases were positive for ENTEROSCREEN- WB™ test. Our results were contrary with the finding of [20] in Pakistan who found that 43 (93.47%) out of 46 culture positive typhoid fever cases were DOT EIA positive. [22] from India found 35 (92.10%) out of 38 culture positive cases to be DOT EIA positive[22]. Contrary findings were also reported by [8] who found that 40 (95.23%) out of 42 culture positive typhoid fever cases to be DOT EIA positive.

In present study, ENTEROSCREEN- WB™ test for its usefulness in clinical typhoid fever cases presenting to our hospital and observed that it has a sensitivity of 50% and specificity of 96%, PPV and NPV of ENTEROSCREEN- WB™ is 66.66% and 92.30%.[23] reported similar results; sensitivity and specificity of (85.45%) and (88.6%) respectively and positive predictive value 51.1%.[23]

CONCLUSIONS

Department wise distribution of blood samples revealed that Medicine department had the highest contribution followed by the paediatrics, obs. & gynae. Male is to Female ratio was approximately 0.8:1 Maximum number of females with enteric fever was reported among the age group between 21-30 years. Fever was the most common clinical presentation followed by body ache and weakness. IgM antibody was detected in major cases indicates recent infection. Taking Widal test results for *S. typhi* isolation as the golden standard in this study, the ENTER-OSCREEN- WB™ showed low sensitivity and higher specificity.

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