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Research Article

A COMPARATIVE STUDY OF LATERAL FLOW IMMUNOASSAY WITH WIDAL TEST IN THE DIAGNOSIS OF ENTERIC FEVER

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ABSTRACT
Enteric fever is an important health problem in developing countries. It is endemic in most parts of India. Overcrowding, poor sanitation contributes to the spread of infection. It may be mild, but sometimes it causes fatal septicaemia. So early diagnosis is important in the management of the cases and also to prevent complications. This study was done to compare Lateral flow immunoassay test with the Widal test in the diagnosis of Enteric fever. A total of 378 clinically suspected Typhoid fever patients were included in the study. Blood samples were collected and processed from these patients. Lateral flow immunoassay was done with rapid Typhoid IgG/IgM test device and Widal test was done with tube agglutination test. Out of 378 blood samples, 77 cases were positive by Widal test and 121 cases were positive by Lateral flow immunoassay test. Widal test and Lateral flow immunoassay test positivity rate in our study is 20.37% and 32.01% respectively. To conclude Lateral flow immunoassay test is rapid, easy, less time consuming, economical and better than widal test in the diagnosis of Enteric fever.

Keywords: *Enteric Fever, Widal Test, Lateral Flow Immunoassay Test*

INTRODUCTION
Enteric fever has been eliminated from the developed countries, mainly as a result of improvements in water supply and sanitation, but it continues to be endemic in the resource limited nations of the world (Ananthanarayan and Paniker, 2013).
The clinical course may vary from mild undifferentiated pyrexia to a rapidly fatal disease. So appropriate laboratory test is essential for the early diagnosis, proper treatment, and to prevent the complications. Enteric fever is usually diagnosed by blood culture, stool culture, bone marrow culture, bile culture and by serological tests (Ananthanarayan and Paniker, 2013).
Blood culture is considered as gold standard in the diagnosis of Enteric fever, which is 90% positive in first week of fever, 75% in second week, 60% in third week and 25% thereafter (Ananthanarayan and Paniker, 2013). Though blood culture is considered as gold standard, but it is time consuming needs minimum of 48 hours, needs experienced laboratory technicians, equipments, costly and not possible in resource limited rural areas.
Stool culture is positive in third week of fever. A positive fecal culture may occur both in carriers and in patients (Ananthanarayan and Paniker, 2013).
Widal test is commonly used method, but it is also time consuming and it has a moderate sensitivity, specificity and nonspecific (Kawano *et al.,* 2007). A fourfold rise in antibody titre in paired sera is diagnostic but it is not possible in all cases especially in rural areas.
Bone marrow culture/Bile culture is expensive, invasive, and difficult to get and also time consuming.
So a rapid, inexpensive and a better test are essential for the proper diagnosis and management of the cases. Immunoassay test doesn't require any specialized laboratory or experienced laboratory personnel. Results can be read within 15 minutes and it can detect anti-salmonella antibodies within 1st week of Enteric fever. The present study was done to compare the lateral flow immunoassay which is rapid and easy, with the widal test in the diagnosis of Enteric fever.

MATERIALS AND METHODS
The present study was conducted in the Department of Microbiology, Dr. B.R. Ambedkar Medical College, Bangalore from July 2014 to December 2014. A total of 378 samples were included in the study.

Widal tube testing was performed on serum sample. A titre of ≥ 160 for "O" agglutinins and a titre of ≥160 for "H" agglutinins was considered positive for typhoid fever in our Medical college hospital. Lateral flow immunoassay test was done on serum by using Rapid Typhoid IgG /IgM test device (CTK Biotech, Inc. CA 92121, USA.) kit. This test is a qualitative antibody detection test with total assay time of 15 minutes. The test cassette consists of 1) a burgundy coloured conjugate pad containing recombinant H antigen and O antigen conjugated with colloidal gold (HO conjugates) and rabbit IgG-gold conjugates. 2) a nitrocellulose membrane strip containing two bands G and M bands and a control band (C band). The M band is precoated with monoclonal anti-human IgM for the detection of IgM anti-S.Typhi and S. Paratyphi. G band is precoated with reagents for the detection of IgG antiboidies. C band is precoated with goat anti rabbit IgG. IgM antibodies if present in patient serum, will bind to HO conjugates. The immunocomplex is then captured on the membrane by the pre coated anti-human IgM antibody, forming a burgundy coloured M band, indicates positive test result. IgG antibodies if present in patient serum, will bind to HO conjugates. The imunocomplex is then captured by the precoated reagents on the membrane, forming an burgundry coloured G band, indicating positive test result. Absence of M and G bands suggests negative test.

RESULTS AND DISCUSSION

Results

A total of 378 clinically suspected Enteric fever cases were included in the study. Out of 378 cases, 167 were male patients, 155 were female patients and 56 were children. The present study includes all the age group. In the present study, 77 cases were positive by widal test and 121 cases were positive by Lateral flow immunoassay test. Widal test and lateral flow immunoassay test positivity rate in our study is 20.37% and 32.01% respectively.

Table 1: Age and sex distribution of Positive cases by widal test

Age in years	Male	Female	Positive by widal test
1-15	31	25	10
16-30	51	48	19
31-45	62	53	27
46-60	38	35	13
>61	16	19	08
Total	198	180	77

Table 2: Age and sex distribution of Positives by Lateral flow immunoassay test:

Age in years	Male	Female	Positive by immunoassay
1-15	31	25	16
16-30	51	48	36
31-45	62	53	43
46-60	38	35	15
>61	16	19	11
Total	198	180	121

Table 3: Positivity rate of widal test and Lateral flow immunoassay test

Test	Positives	Negatives
Widal test	77(20.37%)	301(79.63%)
Lateral flow immunoassay	121(32.01%)	257(67.99%)

Table 4: Comparison of Widal test with Lateral flow immunoassay test

Test	Lateral flow immunoassay Positive	Lateral flow immunoassay Negative	Total
Widal test Positive	69	09	77
Widal test Negative	52	249	301
Total	121	257	

Thus in comparison to widal test, Lateral flow immunoassay test is rapid, better , less time consuming and economical in rural setup.

Table 5: IgM , IgG and combined IgM /IgG Positives detected in Lateral flow immunoassay test.

Antibody type	IgM	IgG	Both IgM/IgG
Number	87	14	20
Percentage(%)	71.90	11.57	16.53

Discussion

Salmonella typhi causes Typhoid fever. The febrile illness often causes severe mental clouding of consciousness and includes headache, anorexia and congestion of mucous membrane which result from toxic effects of endotoxin liberated by the organisms. Symptoms and signs include stepladder pyrexia, hepatosplenomegaly, bradycardia, leucopenia and rose spots on chest and abdomen to fatal septicaemia (Chakraborthy, 2013). Emergence of multidrug resistance strains and chronic carrier state are important cause of morbidity and mortality in typhoid cases (Kumar *et al.,* 2007). Early diagnosis and complete treatments reduce the complications in typhoid fever (Collier). The definitive diagnosis of Enteric fever requires the isolation of organism from blood, bone marrow or rose spots, while isolation of the organism from faces or urine is not that significant as in positive blood or bone marrow culture (Chakraborthy, 2013). Though blood culture is considered as gold standard in the diagnosis of Enteric 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. Widal test is used in many laboratories mainly in developing countries. But it is less sensitive, non-specific, poorly standardized, and interpretation may be difficult in sometimes (Schroeder, 1968). Also, it is difficult to interpret widal test due to sharing of O and H antigens by other Salmonella serotypes and other members of Enterobacteriaceae (Parry *et al.,* 2002). Less sensitivity may be due to pre-existing antibodies in the endemic areas, anamnestic reactions; fimbrial antigens may produce false positive results, treatment with chloramphenicol and in carriers (Ananthanarayan and Paniker, 2013).

So Lateral flow immunoassay test is rapid, easy, reliable and is better test needed for the diagnosis of typhoid fever. The detection of IgM antibodies suggests recent typhoid fever, while the detection of IgG antibodies suggests past/chronic infection. Detection of both IgM and IgG antibodies indicates recent/acute typhoid fever.

In our study, widal test is positive in 77 (20.37%) cases, Immunoassay test is positive in 121 (32.01%) cases. So Immunoassay test more sensitive in diagnosis of typhoid fever compared to widal test. Our study correlates with the study done by Balakrishna *et al.,* (2013) who reported 27.50% positivity rate by Immunoassay test. In a study done by Sanjeev *et al.,* (2013), 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 Typhi-dot positives. All blood culture positives were positive by Typhi-dot. So Typhi-dot is a reliable and an alternative to blood culture in resource limited nations. In a study done by Narayanappa *et al.,* (2009) sensitivity of Typhi-dot M was 92.6% compared to blood culture. Out of 41 blood culture positives, 38 cases were positive by Typhi-dot-M also and 3 cases were negative by Typhi-dot-M. In another study done by Sushma Krishna *et al.,* (2011) out of 61 clinicallly diagnosed Typhoid fever cases, 50 were positive by blood culture and all 50 were positive by Typhoidot (IgM) also. In a study done by Sultana *et*

al., (2012), reported Immunochromatography test positives in 106(70.7%) and 67(44.7%) were positive by Widal test. In another study done by Bulbul Hassan *et al.,* (2013) showed a positivity rate of 90.43% in immunoassay compared to blood culture and widal test.

Conclusion

Though blood culture is gold standard, its nonavailaility in small areas, is time consuming and positive mainly in early period. Widal test used in many laboratories, it is less sensitive and takes 24 hours for the results and gives positive results by the end of 2 weeks or early 3rd week. Immunoassay detects antibodies by the end of 1st week. So immunoassay is better in the diagnosis of Enteric fever in rural setup.

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