Original Research Article

A Hospital-Based Study Determining the Reliability of Immunochromatographic Test (ICT) for the Early Diagnosis of Typhoid Fever: A Comparative Study

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Abstract

Aim: The aim of the present study was to determine the reliability of immunochromatographic test (ICT) for the early diagnosis of typhoid fever when compared to the Widal test.

Methods: This prospective cross-sectional study was undertaken at the Department of Microbiology for the period of 2 years. A total of 200 clinically suspected typhoid fever cases were included in this study. Sample collection and Processing from each patient included in the study, under strict aseptic precautions 3ml venous blood was withdrawn in a well labeled plain vaccutainer tube.

Results: Out of 200 patients 16 (8%) were positive and 184 (92%) were negative by slide Widal test. Out of 200 cases 18 (9%) were positive and 182 (91%) were negative by immunochromatography test. Out of 18 immunochromatography test positive cases, 3 were negative by Widal test. Sensitivity and specificity of immunochromatographic method was 100% and 97.3% respectively considering Widal as standard.

Conclusion: The study concluded that ICT can be used as the suitable method for rapid diagnosis of typhoid fever. Detection of antibody from ICT method is more easy, non-invasive and highly sensitive and specific method. It is useful for small, less equipped as well as for the laboratories with fewer facilities. Since detection rate of antibody by ICT method is quite satisfactory.

Keywords: Immunochromatographic test, Typhoid fever, Widal test

Introduction

India is endemic to Typhoid fever which is a systemic infection with high morbidity and mortality due to overcrowding and poor sanitation in low lying areas. [1,2] Typhoid fever is endemic, and has a reported data for the year 2005 showing 6,53,580 cases and 417 deaths. [3] The annual incidence is as high as 980/100,000 in New Delhi. [4] With the emergence of multi drug resistance in Salmonella typhi, treatment has become very challenging and has further complicated the situation. Due to non-specific clinical history given by the patient, there an urgent need for rapid and reliable laboratory tests for accurate diagnosis. Also rapid diagnosis will help in commencing early treatment with, suitable antimicrobials for rapid recovery and prevention of complication and mortality. Widal test is the most common rapid serological test detecting both IgG and IgM antibodies together for over three decades but it has moderate sensitivity and specificity. Also it detects both the antibodies combined without specifying recent or past infection. On the contrary molecular

test detecting the nucleic acid of the bacilli have a higher sensitivity and specificity but high costs which limits its routine use. [5] The role of the Widal test is also under scrutiny due to its variable sensitivity, specificity, and predictive values that vary between geographical areas. [6]

Patients with typhoid fever carry the bacteria in their bloodstream and intestinal tracts for a long period of time. Salmonella typhi lives only in human beings. A delay in diagnosis and administration of appropriate therapy may significantly increase the risk of adverse outcome and mortality. [7] The O and H antigens are the major antigens used to serotype the Salmonella. The O antigens are Similar to the O antigens of other Enterobacteriaceae but H antigens are different in that they are diphasic. i.e, the H antigens can exist in either of two major antigenic phases. Phase1 (Specific phase) and phase 2 (non-specific phase). O antigen is less immunogenic than H antigen. The titre of O antibody in serum after

infection or immunisation is generally less than that of H antibody. [8] S. typhi produces surface antigen enveloping the O antigen, referred to as Vi antigen. Vi antigen is poorly immunogenic and induces production of low titre of antibody following infection. Vi antibody disappears in early phase of convalescence. Persistence of this antibody indicates the development of the carrier state. [9] In developing countries, facilities for isolation and culture are often not available especially in smaller hospitals. A definitive diagnosis of the disease is required for treatment and to decrease the morbidity, mortality and transmission. Other methods include detection of S. Typhi-specific antibodies by serological test and antigen by immunological test and identification of nucleic acid by Polymerase chain reaction. [10]

The aim of the present study was to determine the reliability of immunochromatographic test (ICT) for the early diagnosis of typhoid fever when compared to the Widal test.

Materials and Methods

This prospective cross sectional study was undertaken at the Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India for the period of 2 years. A total of 200 clinically suspected typhoid fever cases were included in this study. Sample collection and Processing from each patient included in the study, under strict aseptic precautions 3ml venous blood was withdrawn in a well labeled plain vaccutainer tube. The blood was allowed to clot followed by centrifugation of the tube at 3000 rpm for 15 min to separate serum. [11]

Widal slide agglutination test

The sera were subjected to slide agglutination method. The test was performed as per the manufacturer's instructions (Beacon diagnostics Ltd. India). One drop (50 ul) of undiluted test serum was placed on the circles of slide provided in the kit along with positive and negative control serum followed by addition of one drop (50 ul) of antigens "O", "H", "AH", "BH". The contents were mixed with separate applicator stick and the slide was rocked gently for 1 minute.

Interpretation:

Granular agglutination in case of "O" and flocculating agglutination in case of "H" or "AH", or "BH" indicates positive reaction.

Immunochromatographic test

Typhoid IgM/IgG test device is a two site sandwich immunoassay based on the principle of immunochromatography on a membrane. This test is a qualitative antibody detection test with total assay time of 15 minutes. The test device comprises of two membrane assemblies, one for IgM detection and the other for IgG detection. The IgM detection test assembly has a conjugate pad of anti-human IgM colloidal gold conjugate, nitrocellulose membrane predispensed with S.typhi antigen (LPS) at test line region T and a control line protein at control region C. The IgG membrane assembly has a conjugate pad of protein-A colloidal gold conjugate, nitrocellulose membrane predispensed with S.typhi antigen (LPS) at test line region T and a control line protein at control line region C. When test specimen is applied into sample well(S) of the test device, the specimen migrate by the capillary action across the nitrocellulose membrane. If antibody to S. typhi is present in the specimen, it will react to the colloidal gold conjugate and makes an immune complex. The immune complex moves on the membrane and reacts with immobilized antigen of S. typhi resulting in formation of pink/purple line at "T" region. The test contains an internal inbuilt control which should exhibit a pink or purple line at "C" region. The result is invalid if pink/purple line at "C" is invisible. [12] According to instructions given by manufacturer (Oscar Medicare Pvt.Ltd. India), about 5 μl of specimen was added using micropipette into the S+B well or fill the provided disposable plastic dropper with the specimen up to the indicated mark on dropper and add into S+B well. Add 2 drops of buffer into S+B and wait for appearance of pink/purple lines in result window. Results were read within 15 minutes.

Results

Table 1: Serological analysis of sample tested by slide Widal test

Widal test	Number	Percentage
Positive	16	8%
Negative	184	92%

Out of 200 patients 16 (8%) were positive and 184 (92%) were negative by slide Widal test.

Table 2: Serological analysis of sample tested for antibodies of Salmonella by immunochromatography test

Immunochromatography test	Number	Percentage
Positive	18	9%
Negative	182	91%

Out of 200 cases 18 (9%) were positive and 182 (91%) were negative by immunochromatography test.

Table 3: Comparison of Widal test and immunochromatography for the diagnosis of typhoid fever

Test	Result	Widal test		Total	Sensitivity	Specificity
ICT	Positive	15	3	18	100%	97.3%
	Negative	0	182	182		
Total		15	185	200		

Out of 18 immunochromatography test positive cases, 3 were negative by widal test. Sensitivity and specificity of immunochromatographic method was 100% and 97.3% respectively considering Widal as standard.

Discussion

Typhoid fever is a major public health problem associated with significant morbidity and mortality worldwide, causing an estimates between 11 to 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide. [13] It is endemic in the Indian subcontinent including Bangladesh, South-east and Fareast Asia, Africa and South Central America. [14] Salmonella enterica serovar Typhi, is the human-specific causative agent of typhoid fever. The clinical diagnosis of enteric fever is not always accurate because of a wide range of other common fever-causing infections like malaria, dengue fever, leptospirosis, hepatitis, melioidosis and rickettsiosis endemic in India. Accurate diagnosis differentiating typhoid fever from these conditions is often difficult, both in the clinics and laboratory, but is imperative for effective treatment selection. [15] The standard method for the laboratory diagnosis of enteric fever is the isolation of causative organism from specimens especially blood, feces, urine or other body fluids. Isolation of Salmonella typhi from bone marrow is the current gold standard method for confirming a case of typhoid fever. However, this require equipments and trained laboratory personnel seldom found in primary health-care facilities in the developing countries. [16]

Out of 200 patients 16 (8%) were positive and 184 (92%) were negative by slide widal test. Out of 200 cases 18 (9%) were positive and 182 (91%) were negative by immunochromatography test. Out of 18 immunochromatography test positive cases, 3 were negative by widal test. Sensitivity and specificity of immunochromatographic method was 100% and 97.3% respectively considering widal as standard. Widal test is being used as a diagnostic serological test for over a decade, but its low sensitivity and specificity has led to a need of a new specific test. Also, its negativity in early infection and false positivity due to cross reacting antibodies from viral infection, malaria and others has led to its non-reliability. Pre-existing base line antibodies in endemic areas cross reactivity with other Gram negative bacteria and non typhoid salmonella,

anamnestic reactions in unrelated infections and prior TAB or oral typhoid vaccination has also resulted in poor specificity. Due to the various limitations of Widal test in diagnosing infection, came the need of a rapid, reliable and accurate test for diagnosing Typhoid fever. [17] An evaluation of ICT in India was found to be 100% sensitive and 80% specific compared to a blood culture as gold standard. [18] Our study is in near agreement with findings of Jose et al [19] where they found 90% sensitivity and 94.6% specificity of ICT when compared with widal test.

Conclusion

The study concluded that ICT can be used as the suitable method for rapid diagnosis of typhoid fever. Detection of antibody from ICT method is more easy, non-invasive and highly sensitive and specific method. It is useful for small, less equipped as well as for the laboratories with fewer facilities. Since detection rate of antibody by ICT method is quite satisfactory. This test can be applied for field level use, especially in the endemic areas of developing countries like India, even though the standard test is the widal agglutination test.

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