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REVIEW ARTICLE

Different Diagnostic Procedure of Typhoid Fever: A Review Update

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Abstract

Typhoid fever is diagnosed by using a combination of the clinical presentation, the isolation of *Salmonella typhi* from body fluids and by Widal test. In the first week of illness, the diagnosis may be more difficult because in this invasive stage with bacteraemia; the symptoms are those of generalized infections without localizing feature. Cultures of stool, urine, rose spots, bone marrow, gastric and intestinal secretions can all be useful for diagnosis. The efficacy of culture varies with the specimen being tested. In addition, the prehospital antibiotic therapy frequently used in developing countries complicates the isolation of infectious agents from clinical specimens especially from blood. Bone marrow appears to be the most suitable specimen because bone marrow culture has a higher sensitivity than blood culture. The methods of bacterial isolation are inherently slow and take more than 48 hours. That is why, serologic analysis becomes more important. The Widal test has got limitations such as the difficulty in interpretation, the need to demonstrate a fourfold rise after a week and necessity of knowing the endemicity of the area and is useful only in selected patients. The available methods of diagnosis of typhoid fever are either time consuming or are not absolutely reliable. An accurate diagnosis of typhoid at an early stage is important not only for an etiological diagnosis of the patient but also to identify individuals who may serve as a source of infection. The outer membrane protein on the surface of Gram negative bacteria has been considered as important antigens to induce host immune response. Enzyme-linked immunosorbent assays (ELISA) have been considered an alternative approach for the diagnosis of typhoid fever. Therefore, this present review has been designed to describe the different diagnostic procedure of typhoid fever.

Keywords: Typhoid fever, diagnosis, enteric fever, *Salmonella typhi*

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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]. This highly adapted, human-specific pathogen has evolved remarkable mechanisms for persistence in its host that help to ensure its survival and transmission. Typhoid fever is diagnosed by using a combination of the clinical presentation, the isolation of *Salmonella typhi* from body fluids and by Widal test[2]. Laboratory diagnosis of enteric fever is accomplished by culture of the organism from clinical specimens or demonstration of an elevated level of *Salmonella* antibodies, traditionally by the Widal agglutination assay[3].

Variability of Typhoid Fever Diagnosis

The absence of specific symptoms or signs makes the clinical diagnosis of typhoid fever difficult. In areas of endemic disease, a fever without evident cause that lasts more than one week should be considered typhoid until proved otherwise.

Table 1: Non-specific Standard Laboratory Tests for Diagnosis of Typhoid Fever (Parry et al., 2002)

- **Anemia:** moderately anemic
- **ESR:** elevated
- **Platelets:** thrombocytopenia
- **Lymphocytes:** relative lymphopenia
- **Others Hematological changes:**
    - slightly elevated prothrombin time (PT)
    - activated partial thromboplastin time
    - Decreased fibrinogen levels
- **Liver enzymes:** elevated liver transaminase and serum bilirubin levels
- **Serum Electrolytes:**
    - Mild hyponatremia
    - hypokalemia

Blood cultures are the standard diagnostic method; provided a large volume of blood is cultured (15 ml in adults), they are positive in 60-80% of patients with typhoid[4]. Culture of bone marrow is more sensitive. The role of Widal's test is controversial, because the sensitivity, specificity, and predictive values of this widely used test vary considerably among geographic areas. Unfortunately, *S. enterica* serotype typhi shares these antigens with other *Salmonella* serotypes and shares cross-reacting epitopes with other Enterobacteriaceae. Newer serologic tests are being developed but do not yet perform well enough to ensure their widespread adoption[5]. DNA probes and polymerase-chain-reaction protocols have been developed to detect *S. enterica* serotype typhi directly in the blood[6.]

Culture of Different Specimens

Definitive diagnosis of typhoid fever generally requires isolation of the organism from blood, bone marrow, vomitus, fresh stool, or urine. If patients present within the first week of the disease, blood, intestinal secretions, and stool culture results are usually positive in approximately 85-90% of patients with typhoid fever; however, it declines to 20-30% later in the course of the disease[2]. Cultures of stool, urine, rose spots, bone marrow, gastric and intestinal secretions can all be useful for diagnosis[2]. The efficacy of culture varies with the specimen being tested[3]. In addition, the prehospital antibiotic therapy frequently used in developing countries complicates the isolation of infectious agents from clinical specimens especially from blood[3]. Bone marrow appears to be the most suitable specimen because bone marrow culture has a higher sensitivity than blood culture[7]; however, it is a more invasive procedure which renders it impractical for routine use. Isolation of the organism from stool or urine may not be the absolute proof of typhoid fever because faecal or urinary carriers with superimposed other febrile illness may yield positive cultures[8]. The methods of bacterial isolation are inherently slow and take more than 48 hours[9]. That is why, serologic analysis becomes more important.

Blood Culture: The rate of isolation of bacteria from blood approaches 90% in untreated patients in the first week; however,

the figure falls to less than 50% by the third week[10]. The sensitivity of blood culture is higher in the first week of the illness which is reduced by prior use of antibiotics, and increases with the volume of blood cultured and the ratio of blood to broth[11]. Cultures have also been made from the buffy coat of blood[12], streptokinase-treated blood clots[13], intestinal secretions (with the use of a duodenal string capsule)[7], and skin snips of rose spots[14]. Multiple positive blood culture results are 73-97% specific for typhoid fever[15]. Large-volume blood culture and clot culture after serum removal increase sensitivity.

Bone Marrow Aspirate (BMA) Culture: Bone marrow aspirate (BMA) culture is the most sensitive method of isolating *Salmonella typhi*[16]. The sensitivity is 90% at any point in the disease course for as long as 5 days after the initiation of antibiotics[11]. The test is invasive and extremely painful. Clinicians should try to establish the diagnosis with less traumatic means. Bone marrow culture is positive in 80-95% of patients with typhoid, even patients who have been taking antibiotics for several days, regardless of the duration of illness[13]. In addition to that blood cultures are less sensitive than bone marrow cultures because of the lower numbers of microorganisms in blood as compared with bone marrow[11].

Stool and Urine Culture: Stool culture alone is less than 50% sensitive and urine even less so[17]. A single rectal swab culture at hospital admission can be expected to detect *S typhi* in 30-40% of patients[18]. After disease resolution, 3 stool cultures one month apart should be taken to rule out carrier state. The sensitivity of stool culture depends on the amount of feces cultured, and the positivity rate increases with the duration of the illness. Stool cultures are positive in 30% of patients with acute typhoid fever[17]. For the detection of carriers, several samples should be examined because of the irregular nature of shedding. In particular, stool culture results may be positive for several days after *S typhi* ingestion[11]. This is because of inflammation from the intraluminal dendritic cells. Later in the illness, bacteria shed through the gallbladder again because positive stool culture results.

Others Specimens

S typhi has also been isolated from the cerebrospinal fluid, peritoneal fluid, mesenteric lymph nodes, resected intestine, pharynx, tonsils, abscess, bone, and urine, among others[8]. Cultures of punch biopsy samples of rose spots reportedly have a sensitivity of 63% and may show positive results even after antibiotics.

Serological Diagnosis of Typhoid Fever

Widal test

The Widal test has got limitations such as the difficulty in interpretation, the need to demonstrate a fourfold rise after a week and necessity of knowing the endemicity of the area[19]. A fourfold rise in the Widal assay titre is therefore generally required for a definite serological diagnosis, and a second serum sample is rarely obtained in regions where enteric fever is a major concern[20]. It is indicative of typhoid fever in only 40-60% of patients at the time of admission[21]. Test for the presence of *Salmonella* antibodies in the patients' serum may be of value in the diagnosis of enteric fever. The patients' serum is tested by agglutination method for its antibody titre against O, H and Vi suspensions of enteric fever organisms, like *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. The test results are difficult to interpret in areas where typhoid and other Salmonelliosis are endemic[22]. The test is most reliable if the interpretation is made against background of baseline agglutinin titer in normal individual and in non-typhoidal fevers common in the region[23]. A Vi agglutination reaction has been used to screen for *S. enterica* serotype *typhi* carriers. Its reported sensitivity is 70-80%, with a specificity of 80-95%[24].

Interpretation: Positive result in single test by no means always indicate the presence of enteric fever because both false positive and false negative results are common[19] and in

interpreting them the following points are to be borne in mind: (l) The serum of some normal (uninfected) persons agglutinate *Salmonella* suspensions at dilutions up to about 1 in 80, so that titres can not be taken as significant unless they are greater than 80. (2) Persons who have received TAB vaccine may show high titres of antibodies to each of the Salmonella and only if a marked rise of titre of one serotype is observed the result can be regarded as diagnostically significant. H-agglutinins tend to persist for many months after vaccination but, O-agglutinins tend to disappear sooner e.g. within six months. (3) H antibody titre is extremely variable arid subject to nonspecific rise (anamnestic reaction). H-antibody is used to differentiate *Salmonella* species[25]. (4) Non-specific antigens, such as fimbrial antigen, may be present in test suspensions and then give false positive results by reacting with an agglutinin in the serum of some un-infected individuals. (5) The Widal reaction is positive in many healthy carriers. A negative reaction does not exclude the carrier state, but a positive reaction, particularly a Vi titre of 10 or higher, is said to be helpful for the recognition of the carrier (Old 1996).

Rapid Serological Tests

The available methods of diagnosis of typhoid fever are either time consuming or are not absolutely reliable[26]. Therefore, the development of a simple, rapid, economic, sensitive and reliable diagnostic method for early diagnosis of typhoid fever is imperative in endemic areas[6]. Indirect hemagglutination test (IHA), indirect fluorescent Vi antibody, and indirect enzyme-linked immunosorbent assay (IEIA) for immunoglobulin M (IgM) and IgG antibodies to *Salmonella typhi* polysaccharide are available. Monoclonal antibodies against *Salmonella typhi* flagellin are promising developments. Indirect Immunoflorescence test, Indirect Haemagglutination test, Enzyme Immunoassay (EIA) and counter immunoelectrophoresis (CIEP) have been used. The outer membrane protein on the surface of Gram negative bacteria has been considered as important antigens to induce host immune response[27]. Monoclonal antibody produced against 50 kDa outer membrane protein from *Salmonella typhi* showed no cross reactivity with proteins from other bacteria causing enteric fever and enteric fever like illness[28].

Enzyme-Linked Immunosorbent Assays (ELISA)

Enzyme-linked immunosorbent assays (ELISA) have been considered an alternative approach for the diagnosis of typhoid fever[28]. The IgM response to successfully treated bacterial infections generally persists for only a few weeks or months. Demonstration of IgM antibodies to Salmonella antigen might therefore be of more diagnostic significance in an endemic population than detection of IgG[29]. However, there are theoretical limitations to the indirect ELISA for IgM. If the concentration of specific IgG in a sample is substantially greater than the concentration of IgM, it can produce false negative results by competing for the antigen determinants on the plate. On the other hand, an IgM-class rheumatoid factor that may be present in the sample can react with antigen-IgG complexes and produce a false-positive result.

Dot Enzyme Immunoassay (DOT ElA)

Enzyme immune assay (EIA) using microtiter plates have been used in the diagnosis of typhoid fever[30]. Although the microtiter plate assay has the added advantage of processing many samples at a time with high sensitivity, they do have some limitations. Microtiter plate assays require expensive arid special equipment which restricts its use to the large hospitals and laboratories[28]. DOT Enzyme immunoassay which detects antibodies in patient's sera to antigen dotted on nitrocellulose membrane has been applied in the diagnosis of microbial disease[31]. With this simple yet sensitive method, laboratory results can be interpreted without the use of special equipment[28]. DOT enzyme immunoassay has been introduced for the early and rapid sero-diagnosis of typhoid fever[28]. The discovery of a 50 KDa outer membrane protein specific for *S. typhi* has led to the development of this test[28]

(Ismail et al. 1991a). This is commercially available as DOT-EIA (Typhidot(R), Malaysian Bio-diagnostic research SDN BHD, Kualalumpur, Malaysia). The dot EIA (Typhidot©) is a qualitative antibody detection test which detects serum IgM and IgG antibody separately to a specific 50 KDa outer membrane protein of S. typhi using a single serum specimen. It has been found to be highly sensitive and specific[28]. The test is easy to interpret unlike the Widal test where in one has to be aware of the antigenic titers of the normal population, of the area before it could be correctly interpreted. The test is also not affected by antibiotic intake[32] unlike the Widal test[23]. Furthermore, the lack of cross reactions with other Salmonellae and the negative results obtained in cases of non-typhoidal fevers enhanced the diagnostic value of the DOT EIA making it more specific than the Widal test[28] first studied the DOT EIA for the rapid sero-diagnosis of typhoid fever in Malaysia. When tested among hospitalized febrile children in an endemic area the results showed very little difference in diagnostic sensitivity between Widal and Typhidot (91.1% vs 90.3%). In terms of specificity Typhidot gave better results (91.7% specificity) compared to the Widal test (80.8%) and both showed comparable high negative predictive value (90%). The sensitivity and specificity of DOT EIA was evaluated by[33] among febrile Malaysian children. They found that the sensitivity and specificity of Typhidot was 90.3% and 93.1% respectively and the test had comparable sensitivity but greater specificity than those of the Widal test (91.9% and 80.6% respectively)[34], compared the efficacy of Typhidot with the Widal test. A total of 292 patients sera from suspected enteric fever case were screened by both methods. The results indicated that Typhidot was suggestive in 65% of patients, the Widal in 403%, of these Typhidot yielded IgM anybodies alone in 30,1% of subjects within 1-3 days history of fever[35] reported that there was 100% correlation between EIA positives and blood culture positives. Comparing EIA with the Widal results, they found a correlation of 97.6% and 88.9% for culture positive and culture negative typhoid patients respectively[36] found that the EIA results were either IgM-or IgG was positive, sensitivity was 90%, specificity 91% and negative predictive value 97% with the culture used as gold standard. For IgM positive, specificity was 100%. But the specificity of IgG positive alone was reduced 91% which was probably due to persistence of IgG after acute infection.

DNA testing

Polymerase chain reaction assays for identifying *S typhi* are available in some areas. However, this is used mostly for research since the test is generally too expensive for patients in developing countries.

Imaging Studies

Radiography of the kidneys, ureters, and bladder (KUB) are useful if the clinician suspects bowel perforation. CT scanning or MRI may be warranted to investigate abscesses that may occur in, among other sites, the liver or bones.

Histologic Findings

The hallmark histologic finding in typhoid fever is infiltration of tissues by macrophages (typhoid cells) that contain bacteria, erythrocytes, and degenerated lymphocytes. Aggregates of these macrophages are called typhoid nodules, which are found most commonly in the intestine, mesenteric lymph nodes, spleen, liver, and bone marrow but may be found in the kidneys, testes, and parotid glands. In the intestines, 4 classic pathologic stages occur in the course of infection: (1) hyperplastic changes, (2) necrosis of the intestinal mucosa, (3) sloughing of the mucosa, and (4) the development of ulcers. The spleen is enlarged, red, soft, and congested; its serosal surface may have a fibrinous exudate. Microscopically, the red pulp is congested and contains typhoid nodules. The gallbladder is hyperemic and may show evidence of cholecystitis. A liver biopsy specimen from a person with typhoid often shows cloudy swelling, balloon degeneration with vacuolation of hepatocytes, moderate fatty

change, and focal typhoid nodules. Intact typhoid bacilli can be observed at these sites.

Conclusion

Several tests can be performed to diagnose typhoid fever. DOT EIA is a new serologic test for typhoid fever and it is highly sensitive for the early sero-diagnosis of typhoid fever when the Widal test is often insignificant. It also offers the advantage of specificity and reliability over the Widal test and testing of a single serum specimen is often sufficient. Its high negative predictive value in an endemic area is also an advantage.

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