Enteric Fever in Patients of Lahore: Association of Positive Blood Cultures with the Typhidot Immunoassay Tests and Widal Agglutination

Mahmood ul Hassan*,1, Abdul Rehman Haris2, Amir Hussain3, Mustansar Ali3, Muhammad Hashim Raza3

1Govt College University Faisalabad, Layyah Campus (31200), Pakistan
2Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi (46000), Pakistan
3DHQ Hospital Layyah (31200), Pakistan

Article history:
Received: 2 January, 2019
Accepted: 17 April, 2019
Online: 21 April, 2019

Keywords:
testinal fever, Widal, Typhoid, Blood culture, Salmonella

A B S T R A C T

Objective: Laboratory study to assess the correlation of existing Salmonella blood culture isolates with Widal agglutinin titers and EIA-based Typhidot immunoassay antibodies as the gold standard.

Materials and Methods: This study was conducted at the department of community medicine KEMU Lahore for six months from June 2017 to November 2018. 2704 blood samples were taken for cultural studies and Widal test in a clinical laboratory among people with symptoms of clinical intestinal fever. Of these, 1497 were isolated from Salmonella; The sera of these patients who did not accompany Typhidot requests were also subjected to spot immunoassay with the informed consent required for bleeding. All sera were stored at 40° C until selection.

Results: Blood of 802 men (53.6%) and 695 women (46.4%) gave 61.85% S. typhi (n = 926), 31.26% S. Para typhi-A (n = 468) and 6, 88% S. Para typhi. n = 103) insulation. Broad agglutinins were detected in 473 (31.5%) of these people. Without detectable "O" antibodies, 1:80 "H" titers (n = 264: 17.6%) were most commonly observed in children's sera (n = 112; 7.4%). Widal H with agglutinin "O" was recorded in 209 (13.9%) corresponding positive blood cultures, and 104 (6.9%) gave a titre of 1: 320 or more. A total of 1,024 sera (58.4%) did not have detectable Widal antibody. Typhidot immunoglobulin spots (57.1%), negative in 856 sera, were detected in 641 samples (42.8%). IgG-free IgM stains without detectable IgM, IgG stains without IgG were also observed in 22 sera (1.47%) and samples producing S. Para typhi-A isolate (n = 8) and S. Para typhi-B.

Conclusion: S. Para typhi-A has often grown in the last decade, suggesting incomplete protective coverage, probably with a monovalent vaccine. Antibodies against Widal and Typhidot agglutinins were detected in the serum of patients with Salmonella growing in blood in 31.5% and 42.8%, respectively. Widal may be misinterpreted because of possible "lower" agglutinins that have not been inoculated, and the EIA immunoassay is particularly limited only to S. typhi. An ICT based Salmonella serotype three indicator is desirable.

Introduction

It is said that intestinal fever affects 21.6 million people a year and kills about 600,000 people worldwide every year, but Pakistan, Bangladesh, India, Indonesia, China, Laos, Nepal and Vietnam. In these countries, typhoid fever usually occurs in underdeveloped areas due to lack of sanitation, contaminated food and drinking water, ignorance and low socioeconomic status(1). Pakistan is a hyperendemic area of infection and although the exact incidence is variable, intestinal fever is probably the fourth most common cause of death in this country.

When summer disease occurs, it is more common in cities like Lahore, even after the monsoon rain all year round. In addition, the problem worsened with the emergence of quinolones resistant salmonella strains. Therefore, timely and accurate diagnosis with associated effective antibacterial treatment is important to reduce morbidity and mortality due to complications such as intestinal perforation and bleeding(2,3).

The broad agglutination test is most often used for the retrospective or hypothetical diagnosis of patients with clinically suspected intestinal fever. Georges Widal (1896) continued until the 21st century, since he showed that if sera contain antibodies to lipopolysaccharides and / or H flagella protein antigens, the patient's serum will agglutinate typhoid bacilli or parathyroid glands (4,5). Due to its low cost, speed and simplicity, it is also
widely used in Pakistan, and the establishment of a gold standard blood culture is not yet available in many clinical laboratories, even in such giant facilities as Lahore. An alternative test, the EIA-based Typhi dot immunoassay, introduced in Lahore in 1996, aims to specifically detect IgM and/or IgG in the patient's serum for a 50 kDa immunogenic outer membrane protein that is said to be specific for Salmonella enterica serovar typhi (S. typhi) with nitrocellulose strips (5).

However, if there is a strong clinical suspicion of intestinal fever, Widal is still widely used, and when thought provoking, helps rule out some other febrile diseases that mimic typhoid fever in early symptoms such as dengue and fever malaria (6).

In addition to determining the current prevalence of the three major etiologic serotypes that cause intestinal fever in our environment, this study is apparently a lonely intervention that affects the outcome of the respective vaccines in our use and is being evaluated as reported in the literature (7).

This study evaluates the incidence of Widal agglutinin titers together with Typhidot IgM and IgG antibodies in the blood of patients growing salmonella in culture. This not only gives an idea of antibody levels in patients with a confirmed culture of intestinal fever, but also argues that although antibodies are present in the circulation, they apparently do not interfere with the infectious progression of organisms for some reason.

Materials and Methods

This study was conducted from June 2017 to November 2018. Blood samples were taken from 2704 patients with suspected intestinal fever who were referred to a local special diagnostic laboratory with departments in key areas of Lahore for extensive agglutination and cultivation test. The subjects were divided into 8 groups by age from 2 months to 79 years.

Approximately 5-10 ml of venous blood was collected for blood culture by aseptic adult measurements and venous puncture of 2-3 ml in children. Each sample was quickly inoculated directly into an oxidation signal blood culture system (Basingstoke, UK), which contained a unique broth, enabling the cultivation of a variety of aerobic and anaerobic cultures in one bottle. Each loaded vial was incubated for one hour at 370 ° C. The closed system was then placed on an orbital shaker during the first 24 hours of a 7-day incubation period according to the manufacturer's instructions and regularly checked for a visual indication of a positive sample. The broth / blood mixture was injected into the upper chamber by the gases generated during the growth and replication of the organism in the system. With each positive result, samples were taken directly from the indicator chamber for further testing by subculture in blood agar and EMB medium (Oxoid, UK) and identified using isolated API 20 E. Capsules. Positive growth of Salmonella isolates confirmed serologically by the use of specific antisera (Difco, USA).

An additional ml of blood was also taken to obtain serum from each of the subjects in two serological tests. The conventional Widal tube agglutination test was performed using the Widal antigen (Spectrum Diagnostics, Germany) to detect Salmonella antibodies. A 0.4 ml aliquot of each patient's double diluted serum (1:20 to 1: 320) in 0.9% normal saline was analyzed by adding an equal volume of antigen suspension. Negative saline control was included in each batch. Tubes loaded on a metal mesh were immersed in a water bath at 370 ° C for 1 hour. A possible dilution of typhoid or parathyroid gland titration was 1:80 or higher, showing agglutination.

For Typhidot, the presence of IgM and IgG antibodies was determined by incubating 0.5 ml of test serum and control serum with nitrocellulose strips (Reszon Diagnostics, Selangor, Malaysia) coated with a specific antigen. The antigen-antibody complex was visualized directly by co-incubating the anti-human IgM and IgG peroxidase conjugated strips. After addition of the chromogenic substrate, positive tests were interpreted according to the intensity of the blue color obtained compared to the positive control over a total test time of 3 hours. Descriptive statistics SPSS 17 and Microsoft Excel 2010 were used to analyze the data.

Results

The total number of patients (n = 2,704) who applied for Widal based on blood culture and clinical symptoms was 1,539 men and 1,156 women. Blood from 55.3% (n = 1,497) of these individuals produced Salmonella within 3-5 days after plating. The isolates are S. typhi (n = 926: 61.85), S. paratyphi A (n = 468: 31.26) and S. Para typhi-B (n = 103: 6.88) as shown in Figure 1.1.

Of the 1497 growing blood samples, the three Salmonella serotypes are 802 men (53.6%) and 695 women (46.4%) from 2 months to 79 years (Fig. 1.2). A total of 380 children between the ages of 2 and 16 produced Salmonellae, while 1,117 adult isolating strains against intestinal fever were between 17 and 19 years of age. The number of positive cultures was inversely proportional to the patient's age, decreasing from 328 in group 3 (Fig. 1.3) to 08 in the elderly (group 8). The most positive cases were presented at the age of 20-29 (n = 328). The average age was 23.21 years.

The results of the Widal agglutinin and anti-Typhi dot antibodies assays in Salmonella-producing blood samples are shown in Table 1. Fork agglutinins were detected in 473 (31.5%) sera of these patients. Without significant "O" antibodies, but mainly in pediatric serum (n = 112; 7.4%), titers of 1:80 (n = 264; 17.6%) were observed. "H" was recorded together with "O"
agglutinins in 209 (13.9%) of the respective blood cultures, of which 104 (6.9%) gave a titer of 1:320 or more. No detectable Widal antibodies were found for a total of 1024 serum samples. Typhidot was negative in 856 serum (57.1%). However, test points were detected in 641 samples (42.8%). IgM-free IgM spots (n = 314: 20.9%) were mainly observed as for IgM IgG spots (n = 296: 19.7%). IgM without IgG symptoms was observed in 22 sera (1.47%) and IgG spots without IgM para symptoms and blood samples producing S. Para typhi-A isolate (n = 8) and S. Para typhi-B.

Currently, the incidence of this serotype is estimated as 31.26% in a significant proportion of patients in Lahore. In China, India, Nepal, Thailand and the United States, the incidence of relative paratyphoid fever has also increased which was previously less common and caused mild diseases than typhoid fever. Incomplete protection due to replacement of the bivalent TA vaccine with monovalent vaccines effective only against S. typhi(9).

Men are more likely to be infected than women, which is in line with previous studies, suggesting that this trend remains unchanged, suggesting that men are more likely to be infected by Salmonella(10). The gold standard in the diagnosis of typhoid fever is the isolation of S. typhi and S. Para typhi from patients' body fluids, especially blood and bone marrow(11).

However, blood cultures do not detect 10 to 70% of patients with intestinal fever for a variety of reasons: the most common is the insufficient blood sample, the stage of the disease began during bleeding and the use of an antibiotic before sampling. In addition, culture usually requires 3 to 5 days to achieve results, thus encouraging to start an empirical
prescription. Previous studies have shown that the majority of TYP-CN patients have typhoid fever, but culture avoids them(12).

Therefore, serological information is trusted, and the most commonly used traditional method is broad agglutination with the advantages of low cost and easy conductivity(13). Although culture (blood, bone marrow) remains the gold standard, rapid and accurate laboratory analysis for typhoid fever is clearly needed, because the presentation is poorly identifiable and can vary significantly in patients, especially children(14,15). Widal may be misunderstood because of possible "lower" agglutinins that have not been inoculated and the immunoassay point has limited specificity only for S. typhi. Therefore, an ICT-based indicator of three typical Salmonella serotypes is desirable.

Conclusion

Intestinal fever predominates in Pakistan and is growing in the metropolis, especially affecting schoolchildren and young adults. Salmonella typhi remains the main causative agent of intestinal fever, but the incidence of Salmonella Para typhi A has increased significantly over the past decade. A high-frequency test can indicate any of the three Salmonella serotypes when it is still an economical and easy test for a possible diagnosis of intestinal fever and when it is positive. Typhi dot is expensive, requires experience, and has limited specificity in detecting only S. typhi, but typhoid for 1-3 days has the advantage of providing IgM stains as soon as possible.

Conflict of Interest: This study has no conflict of interest which is declared by authors.

Disclosure: None

Human and Animal Rights: No rights violated.

References