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Typhoid fever: Accuracy In Laboratory Diagnosis by Widal test and Blood culture techniques

Pathology

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ABSTRACT

Background: Salmonella typhi and paratyphi are the causative agents of typhoid fever. Ingestion of contaminated food and water is the root source of transmission. Typhoid is a community health challenge for India, peculiarly with the propagation of antimicrobial resistance. The goal of this study was to compare diagnostic accuracy of typhoid fever by Widal and blood culture techniques in a tertiary care center, at western Maharashtra.

Material and Methods: 27750 Blood samples were withdrawn from patients, visiting Hospital from January 2015 to May 2017. Widal slide agglutination test was used for the determination of antibody titer. An antibody titer of >1/80 was taken as a cut of value to designate infection of typhoid fever. Blood culture technique was done under strict sterilization. The Central clinical laboratory provided the sufficient data regarding age and sex.

Results: Out of 27750 participants, 16882(60.83%) participants were females and 10868 (39.17%) were males. 3124(11.25%) samples were found to be positive for salmonella typhi using the Widal test, blood culture method recorded 1268 (4.56%) cases. Total positive results through both the methods are 4392(15.82%). Most common age group affected was 11-30 years, 1398(27.89%) while 534 cases were seen positive via blood culture technique in the same age group. Total females 2327(8.38%), showed positivity in 1655 (5.96%) by Widal test whereas 672(2.42%) in blood culture technique. 1469 (5.29%) and 596(2.14%) were the positive findings in the males, giving a total of 2065(7.14%). The result of this study shows a significant mean difference (t-value = 2.95, p-value = 0.026) between Widal and Blood culture at 5% level of significance.

Conclusion: Widal test was found to be insensitive in comparison to blood culture techniques. Therefore, it is encouraged that the presumptuously high incidence of the disease using Widal test will be significantly decreased if blood culture technique is routinely followed as a basic line of investigation in suspected cases of typhoid fever.

KEYWORDS:

Diagnostic Accuracy, Blood culture, salmonella, Widal test.

Introduction

Typhoid fever still exists as a major community health issue primarily occurring in developing countries like India, Africa, South and Central America with increasing population, urbanization and improper provision of drinking water, hygienic conditions and health organization [1,2]. Typhoid fever is an acute illness associated with fever caused by Salmonella, a gram-negative bacterium [3]. Serotypes of salmonella includes Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B and Salmonella paratyphi C. [4,5] The only reservoir hosts are the human beings. The root of transmission is through consumption of contaminated food or water with faeces of infected individuals incorporating the bacteria. [6] Worldwide, sixteen million cases are encountered annually with 600,000 cases of mortality. [5] Globally, calculated burden of typhoid fever in year 2010 was 26.9 million. In, India it is a common infectious disease. It is endemic in almost all parts of the country with periodic outbreaks of water borne or food borne diseases. In 1992, about 3,52,980 cases with 735 deaths were reported. The number was 3,57,452 cases and 888 deaths in 1993 whereas in 1994, about 2,78,451 cases and 304 deaths due to typhoid fever were reported in 2008. Case fatality rate caused by typhoid fever has been noted from 1.1% to 2.5 % in last few years. [7,8] The most commonly used diagnostic test is the Widal test, for decades. This test is used to measure agglutinating antibodies against H and O antigens of Salmonella typhi. Although, the noticeable drawback of this test is, cross-reactivity with different bacteria of same genus [9]. Bone marrow technique is considered as the gold standard technique for the isolation and confirmation of Salmonella typhi in a case of typhoid fever; but unfortunately its cumbersome to apply this method in a suspected case of typhoid fever as this method is highly painful and demands equipment as well trained laboratory personnel, which is generally unavailable in the primary health centers in the developing world [10]. The other alternative is blood culture technique practical, but less sensitive. It has its own drawbacks, as it needs 48-72 hours for colony growth. This delay leads to late diagnosis as well unnecessary and irrational usage of antibiotics. [11] This study was carried out to evaluate the sensitivity and specificity of blood culture method against the quick- diagnosing Widal test in the accurate diagnosis of typhoid fever.

Material and Methods:

The study was conducted in the Central Clinical Laboratory of Dr. DY Patil Medical College, Hospital and research Centre, Pune from January 2015 to May 2017. A cross sectional study was designed which employed quantitative methods of data collection. All the patients visiting the fever clinic were involved in the study. Selective age group was not picked up. Individuals of all age's group and sexes were included in the study.

Sampling and sample collection

Already collected samples and their results were noted from the Central Clinical laboratory. The blood was collected aseptically by venepucture technique. Five milliliters of blood collected from each person were tested for Salmonella typhi O and H antibodies and also cultured for Salmonella typhi.

Widal Test: 3-5 mL of blood sample was withdrawn into a sterile test tube and centrifuged for 5 minutes for the separation of serum from the blood. A drop of the serum (0.08mL) was pipette and dropped on a sterile slide in four different parts for Salmonella typhi O and another for Salmonella paratyphi H antigens. Antigens O and H were jolted and dropped into the serum accordingly. It was then mixed and rocked gently for 2 seconds. The results were noted as: $^{1}/_{20}$is negative while $^{1}/_{80}$ – $^{1}/_{360}$is positive.

Blood Culture: Two millimeters of blood sample was withdrawn under strict sterile measures and then inserted into 18mL thioglycolate broth, incubated at 37°C for period of 48 hours and then sub-cultured on Salmonella-Shigella Agar (SSA). Salmonella typhi were recognized on the ground of standard culture, microscopic and biochemical characteristics. If growth was not seen within 7-10 days the inoculated blood culture medium was discarded.

Results

Table 1 shows the age-related prevalence of typhoid fever using the Widal test and blood culture. Out of 27750 individuals sampled, 16882(60.83%) participants were females and 10868 (39.17%) were males. 3124(11.25%) were found positive for Salmonella typhi using Widal test and 1268 (4.56%) through Blood culture respectively. The rate of infection among the females was 1655 (6%) using Widal test and 672(2.42%) using blood culture. Whereas 1469 males were found

positive for typhoid fever through Widal test and 596 showed positivity with blood culture growth. The result shows a significant mean difference (unpaired t-test value = 2.9033, p-value =0.0132, df = 12) between Widal and Blood culture.

Table 1. Prevalence of Typhoid fever using Widal test and Blood culture

Sex	Total number of sample examined in suspicious cases of typhoid fever	Total Number of Positive sample using Widal Test (%)	Total number of sample Number Positive using Blood Culture (%)
Female	16882	1655(5.96%)	672(2.42%)
Male	10868	1469(5.29%)	596(2.14%)
Total	27750	3124(11.25%)	1268(4.56%)

Table 2 shows the prevalence of typhoid fever in relation to age. The most common age group effected was from 11-30 years. The least prevalence of typhoid fever was obtained among the age group above 70 years. The total positive candidates for typhoid through the Widal test were 3124(11.25%) and 1268(4.56%) through blood culture. Sensitivity in terms of ratio of Widal test to blood culture is found to be 2.4:1.

Table 2. Prevalence of Typhoid Fever in Relation to Age

Age Group (years)	Number Of total cases examined in particular age group	Number of cases positive using Widal Test (%)	Number of positive cases using Blood Culture (%)
<10 years	3567	400(11.22%)	186(5.21%)
11-20 years	4510	643(14.25%)	264(5.85%)
21-30 years	5532	755(13.64%)	270(4.88%)
31-40 years	5232	601(11.4%)	202(3.86%)
41-50 years	4786	380(7.93%)	164(3.42%)
50-60 years	2343	216(9.21%)	104(4.43%)
70 above	1780	129(7.24%)	78(4.38%)
Total	27750	3124(11.25%)	1268(4.56%)

Discussion:

Enteric fever is a major health issue encountered in developing nations. The confirmatory diagnosis is subjected to the results of Widal test or blood culture technique. Blood Culture diagnosis of Salmonella typhi has unraveled the insensitivity of Widal agglutination test, which is generally the diagnostic method used in many suspected cases of typhoid fever. For definite and authentic diagnosis of typhoid fever, the blood culture method should be applied wherever indicated. This technique is regarded as the gold standard in the laboratory diagnosis of enteric fever. [13] The present study assessed the results through Widal test and blood culture technique. Out of 27750 samples, 3124(11.25%) showed positivity through Widal test while 1268 (4.56%) showed positive for diagnostic blood culture. The results corresponded with the findings of other studied, 62.5% and 55.0% positive results for enteric fever using Widal and blood culture techniques respectively. [8] false positive results through Widal test may misguide us from the true diagnosis, due to cross-reaction of antigen from similar infections with Salmonella antibody. [15] An incorrect result of rapid diagnostic kits postpones the treatment of genuine infection leading to increased morbidity. Increased demand for Widal test as a rapid diagnosis of enteric fever has created magnified results, since enteric fever and malaria usually mimics clinical features and provide false positive results in even laboratory diagnosis. [16] Therefore recommendation of blood culture technique in highly suspicious cases of typhoid fever should be always done in order to avoid irrational and unnecessary usage of antibiotics.so that clinicians can gave accurate treatment, so that overinflated treatment can be avoided.

5. Conclusion

An authentic and accurate diagnosis of typhoid fever relies on blood culture, bone marrow and stool examination. The results of this study have proved blood culture to be most genuine technique of diagnosing early phase Salmonella typhi infections in the absence of other culture methods. Widal test is found have given almost 1/3rd of false positive results. Therefore completely relying over Widal test by clinicians should be avoided and other diagnostic methods should be implicated for the diagnosis of enteric fever, to discriminate Salmonella infection from mimicking infections.

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