

THE SENSITIVITY OF DIAZO TEST IN THE DIAGNOSIS OF ENTERIC FEVERS

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ABSTRACT

To ascertain the sensitivity of Diazo (chemical) test in comparison to the Widal (serological) test in the diagnosis of enteric fevers, blood specimens from 101 patients suspected of having enteric fevers were collected. 54.5% (55) of the patients were significantly seropositive. Fifteen urine specimens from these 55 seropositive patients were positive for Diazo tests, giving a sensitivity of about 27%. *Salmonella typhi* was the predominant serotype causing typhoid/paratyphoid fevers, followed by *S. paratyphi* A; *S. paratyphi* C and *S. paratyphi* B respectively. Although Diazo test does not appear to be reliable, it could still be useful alongside with Widal agglutination test in endemic rural or urban areas where electricity and facilities are absent or inadequate for routine laboratory investigations.

INTRODUCTION

Isolation of *Salmonella typhi*, *Salmonella paratyphi* A, B, and C remains the confirmatory test for the diagnosis of typhoid fevers. This is usually after a presumptive test which is a serology test known as Widal agglutination test has been carried out and found positive. These enteric fever diseases have clinical features that include fever, chills, diarrhea, headache, anorexia, abdominal pain etc (1; 2).

In developing countries however, and in areas where the disease is endemic, such as Nigeria, a single Widal test on serum during acute illness together with compatible clinical features is used for diagnosis but usually when a baseline titer has been established for the community (3; 4).

Diagnosis of enteric fevers is being made difficult as a result of myriad of problems including indiscriminate use of antibiotics and false positive results. Different comparisons of diagnostic

methods have been made in the past (5). However, this is only where laboratory facilities are available. This has brought to the fore front a need to examine other chemical methods which could be used in rural areas of many economically poor countries where electricity and laboratory facilities may be inadequate or completely absent.

The Diazo test of urine, a chemical test for typhoid illness has elucidated many claims to the level that it is the most valuable single test in the diagnosis of typhoid fever. Some authors have stated that it is 30-90% positive in typhoid cases (6; 7).

It does appear however, that there are no data to portray these claims in this part of central Nigeria. The study was therefore undertaken to provide data on the Diazo test as an alternative or otherwise to Widal test in typhoid endemic rural and even urban areas where laboratory facilities may be inadequate or absent.

MATERIALS AND METHODS

Subjects.

Urine and blood samples were collected from 101 patients clinically diagnosed as having typhoid fevers. These patients were attending Jos University Teaching Hospital (JUTH) and Plateau Hospital, both in Jos metropolis of Plateau State, Nigeria.

Preparation of Diazo Reagent

The Diazo reagent was made from two stock solutions, A and B. Solution A was composed of sulphuric acid (0.5g), concentrated hydrochloric acid (5ml) and distilled water (100ml), while solution B was composed of sodium nitrate (0.5g), and distilled water (100ml). Forty parts of Solution A was mixed with one part of Solution B to make the Diazo reagent (7).

Collection of Urine Sample

Early morning urine specimens of patients were collected using clean-dry sterile universal bottles and then appropriately labeled. Samples were immediately processed, and where this was not possible, they were preserved in the refrigerator at 4c before being processed.

The Diazo Test

Equal amount of the urine specimen was mixed with equal amount of the Diazo reagent and a few drops of 30% ammonium hydroxide was added. This was shaken with a positive result showing a red or pinkish coloration of the urine's froth. A negative test showed no colour change.

Collection of Blood Samples

Blood samples of about 3mls each was collected by venepuncture into clean plain containers and allowed to clot. The blood was then centrifuged for 5 minutes at 3000rpm. The serum was separated using Pasteur pipette. Serum not processed immediately were stored at 2-8c before being processed.

Widal Agglutination Test

Two drops of undiluted serum were placed on 2 circles of the test tile. Serum kept in the refrigerator was first brought to room temperature before used. A drop of the appropriate well shaken suspension of somatic and flagella antigens was added to each circle. The contents of each circle was then mixed with a disposable stirrer and spread over the area. The slide was gently rocked by hand for 2 minutes and observed for agglutination.

Widal Titration Test

The tube agglutination test employed the Cromatest stained bacterial suspension antigens. Ten clean dry tubes for both O and H antigens were placed in a rack and labeled 1-10. Using a pipette, 1.9ml of 0.85% saline was dispensed into tube 1 and 0.1ml of the saline into the remaining tubes. 0.1ml of the patient's serum was dispensed into the 1st tube. This was properly mixed and 1.0ml of the mixture from the 1st tube added into the 2nd tube. The 2nd tube was mixed properly and the serial doubling dilution was continued to the 8th tube to give dilutions of the reciprocals of 20, 40, 80, 160, 320, 640, 1280, and 2560 respectively. Tube 9 contained 1.0ml of the normal saline and 2 drops of the positive control while tube 10 contained 1.0ml of the normal saline only which served as negative control. The tubes were incubated in water bath at 48-50c for 4 hours for somatic antigens and 2 hours for flagella antigens after which tubes were examined macroscopically for agglutination.

RESULTS

There were 54.5% (55) patients with significant Widal agglutination titres (i.e. O \geq 160; H \geq 320) out of the 101 suspected of having typhoid fevers. This was based on taking the normal baseline titres of somatic (O) and flagella (H) antigens as the reciprocals of 40 and 80 respectively (4).

Salmonella typhi was the most prevalent with 69.1% (38) positive cases while 27.3% of those with significant Widal agglutination titres were Diazo positive (Table 1).

Table 1: Significant antibody titer and positive Diazo test in relation to different Salmonella serotypes

| Salmonella group | Widal test | | Diazo test | |
|------------------|-----------------------|------------|------------|------------|
| | No. With signt. Titre | % ab. | +ve | % |
| A | 7 | 12.7 | 2 | 13.3 |
| B | 4 | 7.2 | 1 | 6.7 |
| C | 6 | 10.9 | 1 | 6.7 |
| D | 38 | 69.1 | 11 | 73.3 |
| Total | 55 | 100 | 15 | 100 |

DISCUSSION

The result of the present study showing 54.5% patients who have symptoms of typhoid fevers with significant titres tally with the result of Mandal (8) who reported that 46.9% cases of typhoid showed positive Widal test. Caution should however be taken in relying on Widal test for the diagnosis because it can be non-specific and even give significant reading in healthy carriers, post clinical infections, false positives and immunization with typhoid vaccines.

Our result which showed Diazo test to be sensitive in 27.3% of Widal positive cases does not agree with the those of Manson-Bahr & Apted (6) and Cheesbrough (7) who stated that Diazo test is positive in 80% of typhoid cases within the 5th and 14th day of illness. The difference in result may however not be unrelated to the strength of the Diazo reagent used or possible occurrence of some irregularities during the running of the test. Our result however agrees with that of Boosma (9) who found Diazo test not to be helpful in a prospective study of clinical aspect of typhoid fever carried out in two rural Nigerian hospitals.

The present result and that of others especially that of Onile and Odugbemi (10), showed that *Salmonella typhi* is the dominant serotype that causes typhoid fever in Nigeria and some parts of Africa are not at variance with each other.

The paper concludes by recommending that Diazo test does not appear to be reliable but for quick diagnosis of typhoid fever, it could still be used as an adjunct to Widal agglutination test in rural areas where electricity and laboratory facilities are not available. However, since only about 27% of typhoid cases are sensitive to Diazo test, arrangement should be made immediately to transfer the specimens to locations where facilities are available for the usual confirmatory tests.

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