Use of Diazo reagent as a diagnostic test for typhoid fever in adult Egyptian patients

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Abstract

The goal of this study is to evaluate the efficacy of Diazo reagent as a diagnostic test of Salmonella infection in comparison to Widal test and blood culture in both cases and carriers of infection. 90 individuals with fever and abdominal pain, were grouped into three groups, 30 in each group. Group 1: 30 patients diagnosed as typhoid fever by Widal test, group 2: 30 healthy food handlers who were coming to the hospital for check up to obtain health certificate (carrier group), group 3: 30 individuals were healthy volunteers (control group). All of the three groups were undergone blood culture, Widal test and Diazo test. Diazo test had the highest diagnostic accuracy (97.7%) in comparison to Widal test and blood culture, while Widal test had 80% and blood culture had 70% accuracy in diagnosing typhoid. Diazo test was equal to Widal in diagnosis of carriers.

Keywords: Typhoid fever, diazo test, sensitivity, specificity.

Widal test has only moderate sensitivity and specificity. Despite these limitations the test is useful, particularly in areas that cannot afford the more expensive diagnostic methods (7).

Typhoid fever is difficult to distinguish clinically from other causes of prolonged fever. Countries with high rates of disease often lack the capacity to perform blood cultures to confirm the diagnosis, which may delay the initiation of antimicrobial therapy and lead to increased mortality in patients with typhoid fever. Therefore, developing an inexpensive and rapid diagnostic test for typhoid fever that is both sensitive and specific has become a public health priority (8). Diagnosis of Enteric fever is being made difficult as a result of myriad of problems including indiscriminate use of antibiotics, false positive results, high cost, and time taken for results to arrive and variability of reports. so, Diazo test was the solution to avoid all these problems (6).

Diazo test is a simple non-invasive, inexpensive bedside test to diagnose typhoid fever. Diazo test, originally described by Huckstep in 1962, is a diagnostic tool in the diagnosis of Typhoid fever. It is known that the putrefaction of a protein in the intestine of patients with typhoid fever leads to a breakdown product being excreted in the urine as a phenol-ring compound. This is detected by Diazo test (9).

1. Introduction

Typhoid fever is a potentially fatal multi-systemic infection caused by the bacterium Salmonella Typhi. Its clinical features include fever, chills, diarrhea, headache, anorexia, abdominal pain (1). Humans are the natural host and reservoir (2). Transmission of infection occurs by ingestion of food or water contaminated with faeces. Other established risk factors include recent contact with a typhoid patient or carrier, eating ice cream, flavored iced drinks or food from street vendors, and raw fruit

and vegetables grown in fields fertilized with sewage (3).

The disease remains a serious public health problem in developing countries due to poor sanitation and low socioeconomic status (4). The disease is endemic in South-east Asia and Africa and persists in the Middle East, few southern and eastern European countries and central and South America. The disease carries a significant morbidity and mortality in both pediatric and adult populations (5).

Despite being a global health problem, very little progress has been made in the diagnosis of enteric fever. Diagnosis is still made mostly clinically supported by Widal test, Typhidot M test and very rarely by blood culture. Blood culture is not only the gold standard for diagnosis but also suggests the sensitivity pattern of the organism (6).

measles. E) History of antibiotics in the previous two weeks.

2-Full clinical examination:

General and abdominal examination was done for all patients with special emphasis on temperature chart, hepatomegaly, splenomegaly, rash on neck and abdomen, bronchitic typhoid.

3-Routine Investigations:

- Complete blood picture with differential cell count.
- Urine analysis.
- Malta test.
- Stool analysis.
- Abdominal ultrasound (with special emphasis on liver and spleen whether normal in size or enlarged).

4-Specific investigations:

- Blood culture.
- Widal test.
- Diazo test.

Statistical methodology

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 24. Descriptive statistics were done for parametric quantitative data by mean, standard deviation and minimum & maximum of the range, while they were done for categorical data by number and percentage.

2. Patients and Methods

The study was a randomized prospective single center study in which 90 Egyptian individuals were recruited from Minia Fever Hospital in the period between March and September 2016. Individuals were classified into 3 groups.

2.1 Inclusion criteria:

1. Age: older than18 years.
2. Sex: both genders were included.
3. Patients with fever for 4 days or more.
4. Patients undergoing blood culture and were not on antibiotics two weeks before sampling.

Exclusion criteria:

1) Patients younger than 18 years.
2) Patients with measles, pulmonary T.B, or typhus.
3) Pregnancy
4) Patients with fever of less than 4 days duration.
5) Patients with history of antibiotics intake within the preceding two weeks of presentation.

2.2 All individuals were subjected to:

1-Detailed history taking: A) with special emphasis on epidemiological risk factors for typhoid disease. B) History of recent fever with clinical evaluation (degree, duration, pattern, etc.). C) History of other symptoms of Typhoid Fever e.g: malaise, anorexia, abdominal pain and vomiting, rash, chest and CNS symptoms. D) History of pulmonary T.B, typhus and

Hospital in the period between March and September 2016. Individuals were classified into three groups. Group 1; 30 patients who were diagnosed with typhoid fever. Group 2; 30 healthy non-symptomatic food handlers coming for checkup. Group 3; 30 healthy volunteers. Age of all the studied groups were between 19 and 63 years old with no significant difference between the three groups. Male predominance was noticed because 62 out of 90 were male. The study showed equal distribution between urban and rural residence.

Analyses were done for parametric quantitative data between the three groups using one way ANOVA test followed by post hoc Tukey analysis between each two groups. Analyses were done for qualitative data using Fisher exact test. ROC curve analysis was done to determine AUC, sensitivity, specificity, PPV and NPV of variables predicting cases and carriers. The level of significance was taken at (P value < 0.05).

Results .3

Ninety Egyptian individuals were consecutively included in this study from Minia Fever

:Table (1): Comparison between the studied groups as regard their occupation

Occupation	Group 1 (Cases)	Group 2 (Carriers)	Group 3 (Control)	P value < 0.001*		
				1 vs 2	1 vs 3	2 vs 3
1) workers	17(57%)	0 (0%)	11 (37%)	< 0.001*	< 0.001*	< 0.001*
2) Farmers	9 (30%)	0 (0%)	0 (0%)			
3) House wives	4 (13%)	0 (0%)	0 (0%)			
4) Food handlers	0 (0%)	30 (100%)	0 (0%)			
5) Nurses	0 (0%)	0 (0%)	7 (23%)			
6) Employees	0 (0%)	0 (0%)	12 (40%)			

Table 1 shows that 57% of the diseased patients (group 1) were workers (P value < 0.001), carriers were 100% food handlers and 45% of the volunteers were employees.

Splenomegaly	G1(Cases)	G2(Carriers)	G3(Control)	P value< 0.001*		
				1 vs 2	1 vs 3	2 vs 3
Yes	24(80%)	17 (57%)	0 (0%)	0.095	< 0.001*	<0.001*
No	6 (20%)	13 (43%)	30 (100%)			

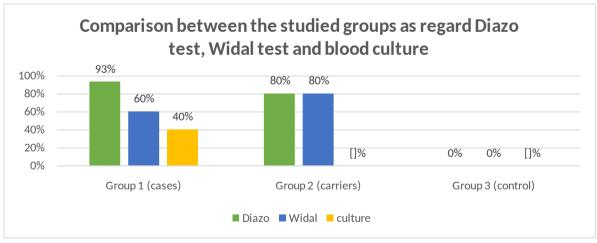Table (2): Comparison between the studied groups as regard Splenomegaly:-

80% of the diseased patients had splenomegaly (P value < 0.001) and 57% of carriers had splenomegaly. Table.2. shows statistically significance between diseased group and control group.

Platelet count	Group 1 (Cases)	Group 2 (Carriers)	Group 3 (Control)	P value <0.001		
				1 vs 2	1 vs 3	2 vs 3
Yes	17(57%)	2 (7%)	0 (0%)	<0.001*	<0.001*	0.232
No	13(43%)	28 (93%)	30(100%)			

Table (3): Comparison between the studied groups as regard thrombocytopenia:

Table 3 shows that the platelet count was decreased in the diseased group in comparison to the carrier group and the control group which is statistically significant. (P value < 0.001*).

Figure (1) shows comparison between the studied groups as regard Diazo test , Widal test and blood culture:

* In total:

1- Group 1 (cases): 93% positive for Diazo vs 60% positive for Widal vs 40% positive for culture.

2- Group 2 (carriers): 80% positive for Diazo vs 80% positive for Widal vs 0% positive for culture.

3- Group 3 (control): 0% for Diazo, Widal and blood culture.

Table (4): Performance of Diazo test in comparison to Widal test and blood culture:

Variable	AUC	P value	Sensitivity	Specificity	PPV	NPV	Accuracy
Widal	0.8	<0.001*	60	100	100	71.4	80%
Culture	0.7	0.008*	40	100	100	62.5	70%
Diazo	0.967	<0.001*	93.3	100	100	93.7	97.7%

Figure (1) and table (4) show Diazo test had the highest diagnostic accuracy (97.7%) with AUC 0.97 in comparison to Widal test and Blood culture. While Widal test had 80% accuracy with AUC 0.8, and blood culture had 70% accuracy with AUC 0.7 in diagnosing typhoid.

Table (5): ROC curve analysis for diagnosis of Carriers;-

Variable	AUC	P value	Sensitivity	Specificity	PPV	NPV	Accuracy
Widal	0.9	<0.001*	80	100	100	83.3	90%
Culture	0.5	1	100	0	50	0	50%
Diazo	0.9	<0.001*	80	100	100	83.3	90%

Table (5) shows no difference between Widal and Diazo test as regard the carriers.

problems (9). Diazo test is a simple non-invasive, inexpensive bedside test to diagnose typhoid fever. Diazo test, originally described by Huckstep in 1962, is a diagnostic tool in the diagnosis of Typhoid fever. It is known that the putrefaction of a protein in the intestine of patients with typhoid fever leads to a breakdown product being excreted in the urine as a phenol-ring compound. This is detected by Diazo test (10).

The current study aimed at identifying the efficacy of Diazo test in the diagnosis of Typhoid fever, 90 persons were included. Subjects were classified into 3 groups: Group 1: 30 cases, Group 2: 30 carriers, Group 3: 30 volunteers as a control group. We excluded patients younger than 18 years, patients with measles , pulmonary T.B, or typhus , patients with fever less than 4 days duration and patients with history of antibiotics intake within the preceding two weeks of presentation.

In our study, distribution of the studied group as regards general data. Average age was 19- 63 years old. Both infected cases and carriers were in the same age range (19-63 vs 19-59) with no significant difference, while the control group were older with the range (19-59). There was a higher mean in the control group (39.3 +/- 11.3) vs 33.7 +/- 10.7 and 30 +/- 7.5 in the cases and carriers groups respectively which were statistically significant. This can be explained by all cases and carriers were in the

4. Discussion

Typhoid fever is a potentially fatal multi-systemic infection caused by the bacterium Salmonella Typhi. Its clinical features include fever, chills, diarrhea, headache, anorexia, abdominal pain (1). Humans are the natural host and reservoir (2). Transmission of infection occurs by ingestion of food or water contaminated with faeces. Other established risk factors include recent contact with a typhoid patient or carrier, eating ice cream, flavoured iced drinks or food from street vendors, and raw fruit and vegetables grown in fields fertilized with sewage (3).

Despite being a global health problem, very little progress has been made in the diagnosis of enteric fever. Diagnosis is still made mostly clinically supported by Widal test, Typhidot M test and very rarely by blood culture. Blood culture is not only the gold standard for diagnosis but also suggests the sensitivity pattern of the organism (6).

Widal test has only moderate sensitivity and specificity. Despite these limitations the test is useful, particularly in areas that cannot afford the more expensive diagnostic methods (7). Diagnosis of Enteric fever is being made difficult as a result of myriad of problems including indiscriminate use of antibiotics, false positive results, high cost, and time taken for results to arrive and variability of reports. So, Diazo test was the solution to avoid all these

disagreement might be due to the lower number of cases in our study**.** On the other hand, results from (16) study showed that: rural areas were around 2-3 times higher among cases and carriers than urban areas**.**

The majority of infected cases were workers with significant difference. This can also be explained by those groups of patients are more subjected to the outdoor food intake. The control group showed an equal job distribution between workers and employees. The carrier group was food handlers.

Our study is in agreement with the study done by (17) which was done on 209 food handlers, and among them 199 were positive for salmonella (carriers). The study concluded that carrier rate of typhoid in food handlers working in different food streets was very high. These food handlers might be contributing to the high endemicity of typhoid fever.

As regard the clinical findings; it was interesting to find that hepatomegaly was detected in only 40% of infected patients and 27% of carrier group. On the other hand, splenomegaly was detected in 80% of infected group and 57% of carrier group. Both hepatomegaly and splenomegaly were absent in the control group.

*This is in agreement with a study by (10) *in* which splenomegaly was found in 37% of cases and hepatomegaly was found in 17% with typhoid fever.

working age group and were exposed to the continuous outdoor food intake. These findings were similar to a study (10) which was done on 30 patients aged > 15 years; 86% were clustered around age 15- 60 years old.

On the other hand*,* (11) studied a group of 455 infected subjects, and only 122 of them (29%) ranged between 18 and 60 years old, while the majority of cases (71%) ranged between (0 - 4) years old. males were 62(69%), while females were 28 (31%), P value = 0.016*. Male gender was predominant in the carrier group and also in the infected group. This study was in agreement with a study (10), which revealed that 17 subjects out of 30 were males (57%) and the remaining 13 were females (43%)

However this was not in agreement with the study (12) ; which was done on 119 subjects. 70 of them were male (58.8%) and 49 were females (41.2%)**.** A study of (13) which was done on 46 subjects and revealed that male: female ratio was 1:1**.**

There is no significant difference as regard the residence in all the groups. Our study is in agreement with a study done by (14)*;* which was done on 455 cases and carriers and illustrated that no significant difference between urban and rural areas**.** But not in line with results from (15) which demonstrated that typhoid cases were clustered in urban areas in which was done on 2570 cases. However, this

Our study has higher results than those done by (23) in which sensitivity of Diazo test was 86.7% and specificity was 85.7%.

A study was done by (23) on 112 cases and carriers revealed that the sensitivity and specificity of Diazo test was lower than Widal test but higher than Blood culture. This study is different from our study which stated that Diazo test has the highest sensitivity (93.3%) while Widal test had 60% sensitivity and culture was of 40%, and the whole three tests had the same specificity of 100%.

Conclusion and Recommendations .5

Salmonella carriers are significantly common in males than females. On the other hand, typhoid fever disease showed no gender variation. Both occur in the middle age group range. Workers are more subjected to typhoid infection may be due to continuous outdoor food intake. Clinically, splenomegaly is an evident sign in both typhoid ever disease and salmonella infection carriers. Decreased platelets count is an evident laboratory investigation.

Diazo and Widal tests are equally effective in diagnosis of Salmonella in carriers, but **Diazo** is the best diagnostic test of Typhoid fever infection. Diazo test is simple, with highest accuracy, easy and cheap test.

Splenomegaly in a clinically normal individual must be tested for salmonella

* Those results were also similar to a study done by (18) which included 350 cases and carriers. All of them had splenomegaly (100%) and hepatomegaly was found in 31.4% of cases. This is not in line with the study performed by (19) that stated that hepatomegaly is quiet equal in percentage with splenomegaly (44 vs 40) respectively.

Concerning the laboratory data: In our study; hemoglobin and total leucocytic count are within normal range for all the included subjects, while, platelet count was decreased in most cases of the infected group in comparison to the carriers and control groups.

These results were in agreement with a study done by (20) which stated that 100% typhoid cases had thrombocytopenia. This was also confirmed by results from (21) and the study by (13).

As regards diagnosis of cases: This study showed that Diazo test had the highest degree of sensitivity in Typhoid cases. This study showed that Diazo test had the highest accuracy (97.7%) which was higher in comparison to Widal test and blood culture (97.7% vs 80% vs 70% respectively). Our results are similar to those from (22) that showed a sensitivity of 81% and specificity of 90% and another study done by (23) that showed a sensitivity of 92% and specificity of 83%.

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infection and to be considered as a carrier. Large cohort including variable age groups, jobs, subjects working in food related jobs compared with food unrelated jobs must be performed.

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