Australian Journal of Basic and Applied Sciences, 4(4): 609-614, 2010
ISSN 1991-8178

A Comparative Study of Blood Culture and Antibody Response with the Duration of Illness in Diagnosis of Typhoid Fever

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Abstract: Diagnosis of typhoid fever is difficult because it is non-specific symptoms and signs with those of other acute febrile illness. Blood cultures, and antibody levels of O& H, were evaluated, in the diagnosis of typhoid fever in Egyptian hospitals. Patients were divided into three groups: (I) 254 with blood culture-confirmed typhoid, (II) 3426 febrile patients with negative blood cultures, and (III) 176 with other bacteremic pathogens. In-group (I), 77% had (O&H) antibody titers ≥ 1:160, compared to 12% of group (II), and 8% of group (III). Among group (1), 73% of patients were culture positive for typhoid, in the first week after symptom onset as compared to 89% in the second week and later. The Widal titer of ≥ 1/160 had sensitivity of 77 %, and specificity of 89%. In the first week the sensitivity of confirmed blood cultures, and antibody titer of ≥ 1/160, are almost the same, while in the second week and after, the positive Widal serology is much higher than the confirmed blood cultures. The O and H antibody titers of ≥ 1/160 of single Widal test is useful in the diagnosis of typhoid fever.

Key wards: Evaluation of blood culture and prevalence of O&H antibodies in typhoid diagnosis, Widal and diagnosis of typhoid fever, Widal and blood culture tests in hospitals

INTRODUCTION

Typhoid fever (TF), continues to be an important health problem in many developing countries (Hatta *et al*, 2002), with an approximately 16 million cases occurring worldwide annually (Parry, 2004). In Egypt the estimated incidence of typhoid fever is 59/100,000 persons / year (Srikantiah *et al*, 2006). Since the signs and symptoms of the disease are nonspecific, a specific diagnosis is difficult (Hoffman *et al*, 1984). A definite diagnosis is usually obtain when the etiologic agent *Salmonella enterica* serotype Typhi, is isolated from bone marrow or blood cultures (Wain *et al*, 1998; Willke *et al*, 2002). However, blood culture capacities are often not available in endemic areas. The Widal test has been in use for more than a century (Olopoenia & King, 2000) as an aid in the diagnosis of TF. It could be used for the diagnosis of TF of patients who are culture negative, or in regions where bacterial culturing facilities are not available (Hoa *et al*, 1998). The value of the test has been debated for as many years as it has been available (Grunbaum 1896; Jesudason *et al*, 2002). After antibiotic treatment, bacteria might not be isolated from blood cultures and the diagnosis relies on a combination of clinical and epidemiological features. In such cases, the detection of antibodies to ST might be the only way of confirming the clinical diagnosis (Chart *et al*, 2000).

MATERIALS AND METHODS

Patients in fever hospitals with suspected typhoid fever were enrolled. Three groups of patients were classified: Group I, 254 Patients with blood culture-confirmed TF, Group II, 3426 febrile patients with negative blood cultures, who had a febrile illness other than TF, and Group III, 176 patients with blood had other bacterial pathogens (*Brucella spp* 113, *Streptococcus spp*. 7, *Salmonella spp*. 3, *Escherichia coli* 6, *Pseudomonas aeruginosa* 1, *Staphylococcus aureus* 14, *Enterobacter sp*. 3, *Viridans streptococcus* 20, *Enterococcus spp*. 5, and mixed infections 4). Any person > 1 year of age, with a temperature on admission

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of > 38ºC, or history of fever of > 2 days duration, and no identified cause of fever. Blood samples (5-10 ml) were drawn into a biphasic blood culture bottle (PML Microbiological, Wilsonville, Oregon). Blood cultures were placed in the incubator at 37°C, and remained for 2 weeks. In addition, 2-3 ml of blood was collected for uses requiring serum.

Widal Test:

Commercially available *S*. Typhi somatic (O) and flagella (H) antigens were used. Single Widal tube test was performed following manufacturer instructions (SA Scientific Inc., San Antonio, TX). Serum samples were serially diluted up to 1/2560 in normal saline.

Statistical Analysis:

Sensitivity: (true positive rate, a/a+c), **Specificity:** (true negative rate, d/d+b), **Positive Predictive Value:** (PPV, a/a+b), and **Negative Predictive Value:** (NPV, d/d+c) were calculated. Where a= Positive culture, and positive Widal test, b= Negative culture, but positive Widal test, c= Positive culture, but negative Widal test, and d= Negative culture, and negative Widal test.

Results:

All enrolled patients (3856) were > one year old, 43% were females. Most of cases occurred in children aged 6-10 years while 8% of cases were in children from 1-5 years of age (Figure 1). The median age of positive typhoid cases is 10 years old. Sensitivity, specificity, PPV, and NPV of Widal, are shown in (Table 1). Among the blood culture confirmed typhoid cases, 27% showed no response to either antibody, during the first week, 11% showed no response of either antibody during the second week, and after the second week (Table 2). The rate of recovery of bacteria, is decreased with the duration of symptoms prior to blood culturing, and went from 72% in the first week, to 21% in patients with symptoms in the 2nd week, and 7% in patients with symptoms >15 days (Figure 3). The O antibody was present at a titer of ≥ 160 in 113/182 (62%) of culture positive cases in the first week, in 35/54 (65%) in the second week, and in 9/18 (50%) after the 2nd week (Figure 2). While H antibody was present in 102/182(56%) of culture positive cases in the first week, 36/54(67%) in the second week, and 14/18 (78%) after the 2nd week (Table 2). However, (Fig. 2) showed that O & H antibody titers ≥ 1/160 could appear at the second day since onset of symptoms, the maximum of O titer showed high levels at the 2nd and 4th day and then declined, while the maximum H titers showed the highest level at the 5th day and then declined. Both O&H antibodies together were maximum on the 5th day. On the other hand, the confirmed blood cultures of TF cases and the positive Widal serology (≥ 1/160) almost have the same distribution in the first week, while in the second week and after that, the positive Widal serology is much higher than the confirmed blood cultures (Fig. 3).

Table 1: The sensitivity, specificity, PPV, and NPV of (1/160) titers of Widal test (O and/or H) for diagnosis of TF.

Clinical groups (n)	Widal titer ≥ 1/160 (N, %)
Group I (254) Blood culture positive for ST.	196 (77%)
Group II (3426) Negative blood cultures	400 (12%)
Group III (176) Blood cultures positive for other pathogens.	14 (8%)
Sensitivity:	77%
Specificity:	89%
PPV:	32%
NPV:	98%

Table 2: The distribution of O&H antibody titers (≥ 1/160) and sensitivity of Widal test among culture confirmed TF cases (n=254) by time since onset of symptoms

Widal values for O&/ H antibody titers, and	First week (n=182)	Second week (n=54)	After 2nd week (n=18)
Positive O titer only	31 (17%)	12 (22%)	2 (11%)
Positive H titer only	20 (11%)	13 (24%)	7(39%)
Positive O&H	82 (45%)	23 (43%)	7 (39%)
Total positives	133 (73%)	48 (89%)	16 (89%)
Sensitivity	73%	89%	89%

Discussion:

The definite diagnosis of TF is usually obtained when Salmonella *enterica* is isolated from bone marrow or blood cultures. Since facilities, are not available, particularly for the blood culture in the majority of

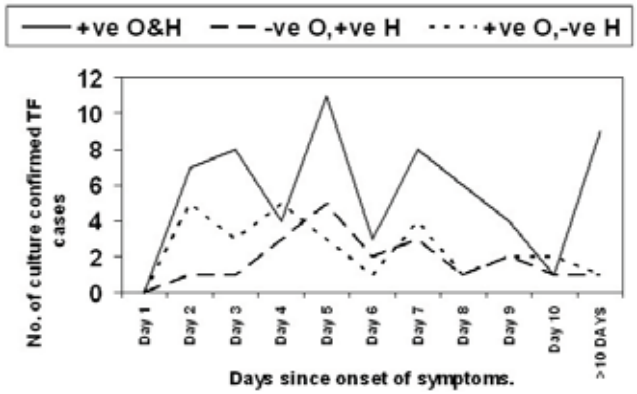

Fig. 1: The distribution of Widal titers (O&H) ≥ 1/160, among blood culture confirmed TF cases by days since onset of symptoms.

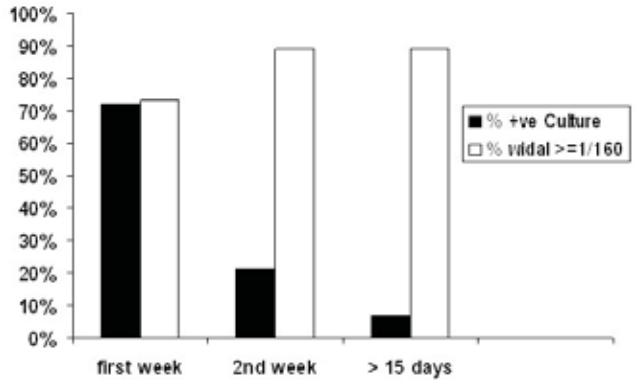

Fig. 2: The percent distribution of confirmed culture TF cases with positive Widal serology by time since onset of symptoms.

developing countries, Widal test remains a valuable tool, and the most useful complimentary test for clinical diagnosis of TF (Senewiratne & Senewiratne 1977; Levine *et al* 1978).

In Egypt, Widal test is still used in the diagnosis of TF, and this study is the first one done to develop local recommendations for the interpretation of Widal results, in relation to blood culture to help physicians and lab personnel interpret the Widal results.

The levels of antibodies detectable in the non-infected populations of different areas vary considerably (Anonymous, 1978; Pang & Puthucheary, 1983; Pu &Huang, 1985; Chongsa-nguan *et al* 1989; Hamze *et al* 1998; House *et al* 2001; Olsen *et al* 2004). The variations of the immune response depend on the degree to which the disease is endemic in each area, duration of illness, dose of infection, pathogenicity, and prior antibiotic use (Hatta *et al* 2002). Most of cases occurred in children aged 6-10 years as described by (Walia *et al* 2006). There were numerous reports on the single Widal test, but no consensus as to its diagnostic value in regions in which typhoid is endemic (Schroeder 1968). In some reports Widal, results may lack specificity particularly in a community with endemic typhoid fever (Pang & Puthucheary 1983) like Fayoum Governorate. The high NPV (98%) indicates that Widal is useful in countries such as Egypt, where other diseases of similar clinical presentation are common. Based on our results, a negative result would have a good predictive value for the absence of the disease, but a positive result would have a very low predictive value for typhoid fever (Somerville *et al* 1981). However, the sensitivity was highest (89%) in the second week of illness, as in many assays previously reported (Prokopec *et al* 1991).

In the present study, the detection of H antibody is not less important than the O antibody, although the earliest serological response in acute typhoid fever is a rise in the titer of the O antibody, with an elevation of the H antibody titer developing more slowly but persisting longer than the O antibody cutoff titer as described before (Anonymous 1978; Olopoenia & King 2000; Willke *et al* 2002). The antibody titers of O

or H antibodies were 62% and 56% respectively in agreement with positive culture typhoid cases, while the two antibodies were found in 73% of cases in the first week since onset of symptoms and can be helpful in diagnosis of the illness (Parry *et al* 1999), (Table 2). This shows that the two antibodies should be considered, as reported before (Coovadia *et al* 1986). Either O or H titers can reach diagnostic level (≥ 1/160) starting from the 2nd day (Fig.2) but not on days 6-8 after the onset of disease as described by (Ismail TF, 2006), and 73% of patients with typhoid fever had diagnostic titers for both O& H during the first week after symptoms appeared. Patients investigated in the first week of the disease might be thought to have contributed to the proportion of patients with negative results (Sharma *et al* 1993; Saha *et al* 1996). In our study, 56% had either H antibody titer only or H-titer & O titer of acute cases in the first week (Table 2). That is because of previous or multiple exposures to typhoid disease in an endemic area like Fayoum Governorate. In addition, some of patients with blood culture-positive of TF had no detectable O or H antibodies at a titer of ≥ 1/160. Although these patients may have had antibodies at a lower titer, they may have a negative Widal test throughout the course of their illness (Reynolds *et al* 1970; Pang & Puthucheary 1983, 1989; Coovadia *et al* 1986; Saha *et al* 1996; Parry *et al* 1999). This lack of antibody response among patients with blood culture-positive typhoid fever has been attributed to undefined host or bacterial factors or prior antibiotic treatment (Anonymous 1978). In the view of the high specificity (89%) and moderate sensitivity (77%) in our study, the Widal test still has value as complimentary test for clinical diagnosis of TF (Jesudason *et al* 2002; Itah & Akpan 2004; Smith *et al* 2004), or in patients who have clinical typhoid fever, but are culture negative or in regions where bacterial culturing facilities are not available (Chongsa-nguan *et al* 1989; House *et al* 2001; Handojo *et al* 2004). Confirmed blood cultures of TF cases and the positive Widal serology (≥ 1/160) have almost the same percentage agreement in the first week since onset of symptoms, while in the second week and after that, the positive Widal serology is much higher than the confirmed blood cultures. As cultures are time consuming, increased efforts should be made to develop other more rapid, sensitive and specific test (Parry *et al* 1999; Arya *et al* 2005; Watt *et al* 2005) to supplement clinical and blood culture data.

Conclusions:

The antibody titers of O&H ≥ 1/160 of a single Widal test is useful in the diagnosis of typhoid fever in Egypt. Since the Widal test is easy to perform inexpensive, and requires no specific equipment and minimal training of staff, it will be a useful complimentary test for clinical diagnosis of TF, or in patients who have clinical typhoid fever, but are culture negative or in regions where bacterial culturing facilities are not available.

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