

ELISA TECHNIQUE FOR DETECTION OF SALMONELLA ANTIGEN IN CHRONIC SCHISTOSOMIASIS

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ABSTRACT

Different techniques (Blood culture, Widal test and ELISA) were carried out to detect the presence of *Salmonella* organisms and/or their antigens or antibodies in order to evaluate the role of such organisms as a cause of the fever frequently encountered in bilharzial hepatosplenomegaly. This study was done on 68 cases, classified into 3 groups: group I, bilharzial hepatosplenomegaly with fever, group II, bilharzial hepatosplenomegaly without fever and group III, clinical enteric fever. Comparison of results indicated that *Salmonella* somatic antigen detection by ELISA was more sensitive than by Widal test (100% vs. 50%) in group I. On the other hand specificity of Widal test (60%, gp. I, 94.4 gp. II, 77.8% gp. III) was greater than ELISA (45% gp. I, 77.8% gp. II, 66.6 gp. III), using blood culture as gold standard. ELISA technique is more sensitive but less specific than Widal test in detection of *Salmonella* infection.

INTRODUCTION

Chronic salmonellosis caused by a variety of *Salmonella* species, particularly *S. typhi* and *S. paratyphi* A often complicates advanced hepatosplenic schistosomiasis⁽¹⁾. Among the various immunological tests for the detection of antibodies to *S. typhi* and *S. paratyphi* is the Widal test which is inexpensive and simple. However, numerous studies have cast serious doubts on the value of the Widal test in the diagnosis of enteric fever especially in endemic areas, because of false positive results⁽²⁾.

Because of the difficulties encountered in antibody testing for salmonellosis, especially in relation to non-specific cross-reaction,

antigen detection tests have been sought. The detection of antigens in body fluids has been introduced to overcome the difficulties encountered in culture techniques and antibody detection in the diagnosis of Salmonella infection, especially typhoid fever⁽³⁾.

This study aims at evaluating of the role of Salmonella organisms as a cause of prolonged intermittent fever frequently encountered with endemic schistosomiasis. Besides, an attempt was made to evaluate the efficacy of enzyme linked immunosorbent assay (ELISA) technique in the detection of Salmonella antigens for the rapid and early diagnosis of cases with enteric fever and its comparison with conventional culture methods and the Widal test.

MATERIALS AND METHODS

Cases : The study was conducted on 68 cases, classified into three groups as follows:

Group I : 22 patients diagnosed as bilharzial hepatosplenomegaly with history of recurrent attacks of fever.

Group II : 21 patients diagnosed as bilharzial hepatosplenomegaly without history of fever. Patients of group I & II were admitted in the Department of Tropical Medicine, Zagazig University Hospitals.

Group III : 15 patients diagnosed as enteric fever admitted in Zagazig Fever Hospital.

A control Group of 10 healthy controls with neither history of bilharziasis nor prolonged fever and were selected from the medical and paramedical staff.

All patients and control groups were subjected to complete clinical assessment, urine and stool analysis, blood picture, abdominal sonography (only in groups I & II), sigmoidoscopy, and rectal snip biopsy for detection of bilharzial ova as well as liver function tests including serum bilirubin, GOT, GPT, albumin and total protein using BioMerieux test kit (France).

Microbiological Investigations :

a. Blood cultures (BioMerieux) were done for all cases. Any growth recorded was subcultured on MacConkey's agar plates (Oxoid). Suspected colonies on MacConkey's plates were identified morphologically, microscopically (Gram stained smears) and biochemically by using API 20 E system for Enterobacteriaceae (Analytap products-USA).

b. Widal test for Salmonella antibody detection was done in all cases (Behring-Germany).

c. Microwell ELISA technique for Salmonella antigen detection. The ELISA screening kit for Salmonella species was used according to the instructions of the manufacturer (Behring-Germany).

Statistical Analysis :

The sensitivity and specificity of ELISA and Widal were performed according to standardized statistical methods⁽⁴⁾.

$$\text{Sensitivity} = \frac{\text{True positive cases}}{\text{True positive cases} + \text{False negative cases}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative cases}}{\text{False positive cases} + \text{True negative cases}} \times 100.$$

RESULTS

In group I only two patients (9.1%) were positive for Salmonella organisms by blood culture, and 9 patients (40.9%) were positive for Widal test, while 13 patients (59.1%) were positive for Salmonella somatic antigen by ELISA in their sera. In group II, three patients (14.3%) were positive for Salmonella organisms by each of blood culture and Widal test. ELISA test for Salmonella antigen detection was positive in six patients (28.6%). In group III, six patients (40%) were positive for Salmonella organisms by blood culture, while 7 patients (46.7%) were positive for Widal test, and 8 patients (53.3%)

were positive for *Salmonella* antigen in their sera by ELSIA. The healthy controls showed a negative result for all tests (Blood culture, Widal test and ELISA).

ELISL was twice as sensitive as Widal test (100% vs 50%), while the specificity of Widal test (60%) was greater than ELISA (45%) in group I, table 2.

The sensitivity of Widal test and ELISA was the same in group II (66.6%) and in group III (83.3%). While the specificity was greater in Widal test (94.4% gp. II, 77.8% gp. III) than ELISA (77.8% gp. II, 66.6% gp. III), tables 3 & 4.

DISCUSSION

The association of hepatic cirrhosis and bacteremia due to enteric bacteria was reported⁽⁵⁾. Abdel Wahab,⁽⁶⁾ reported that *Salmonella* infection in patients with schistosomiasis assume a different clinical picture and course from that observed with the same infection in patients without schistosomiasis . In the former patients, a history of prolonged fever before admission to hospital is common. It is assumed that schistosomal hepatic fibrosis is the cause of frequent association of septicaemia observed in patients with prolonged fever. Wyke et al,⁽⁷⁾ reported that defects of serum opsonization, complement activity and polymorphonuclear leuckocytes function may be among the causes of increased susceptibility to bacterial infection in patients with chronic liver disease. Moreover bilhariza worm act as a carrier for *Salmonella* organism in cases of hepatosplenomegaly complicated with chronic salmonellosis ⁽⁸⁾.

Benavente et al,⁽⁹⁾ reported that the isolation rate of *Salmonella* species was 45%, and Rubin et al,⁽¹⁰⁾ recorded an incidence of 48.3%. This agrees with our results, where blood cultures were positive for *Salmonella* species in 6 out 15 patients (40%) in group III. The relatively low isolation rate detected may be explained by unperiscribed antibiotic adminstration which could decrease the

Table 1: Positive salmonella infection as detected by different techniques in clinical cases (No.=68)

Cases	No.	Blood Culture		Widal Test		ELISA	
		No	%	No	%	No	%
Group I, hepatosplenomegaly with fever	22	2	9.1	9	40.9	13	59.1
Group II, hepatosplenomegaly without fever	21	3	14.3	3	14.3	6	28.6
Group III, clinical enteric fever	15	6	40	7	46.7	8	53.3
Control	10	0	0	0	0	0	0

Table 2: Sensitivity & specificity of Widal test and ELISA in group 1 (NO.=22)

Test		Blood culture		Analysis	
		+ Ve	- Ve	Sensitivity	Specificity
Widal	+ Ve	1	8	50%	60%
	- Ve	1	12		
ELISA	+Ve	2	11	100%	45%
	- Ve	0	9		

Table 3: Sensitivity & specificity of Widal test and ELISA in group II (NO.=21)

Test	Blood culture		Analysis	
	+ Ve	- Ve	Sensitivity	Specificity
Widal	+ Ve	2	66.6%	94.4%
	- Ve	1		
ELISA	+Ve	2	66.6%	77.8%
	- Ve	4		
		1	14	

Table 4: Sensitivity & specificity of Widal test and ELISA in group III (No.=15)

Test	Blood culture		Analysis	
	+ Ve	- Ve	Sensitivity	Specificity
Widal	+ Ve	5	83.3%	77.8%
	- Ve	2		
ELISA	+Ve	5	83.3%	66.6%
	- Ve	3		
		1	6	

concentration of organisms in blood which was directly reflected on blood culture results (11).

The controversy about Widal test as a dependable technique is clearly seen in literature. Coovadia et al,⁽¹²⁾ found anti-O agglutinins at significant titres in 71% of suspected typhoid cases, while Pang and Puthucheary⁽¹³⁾ found that 93% of proven typhoid cases have significant anti-O agglutinins. On the other hand a moderately low percentages of anti-O agglutinins in serum samples of typhoid cases accounting for 58% and 60% were obtained by Abraham et al⁽¹⁴⁾, and Tantivanich et al⁽¹⁵⁾, respectively. Lower results (39.6%) were obtained by Abdel-Fattah et al⁽¹¹⁾.

In the present study it was found that diagnostic titre of anti-O agglutinins was obtained in 9 out of 22 patients (40.9%) in group I, and in 7 out of 15 patients (46.7%) in group III, but only in three patients (14.5%) in group II.

The relative low positivity of the Widal test in our study may be accounted for by the widespread practice of empirical usage of antibiotics before seeking medical advice which could delay and even inhibit agglutinin formation.

EL-Rooby⁽¹⁾ reported that the cause why Widal agglutination test usually shows no or slight elevation of Salmonella titres in cases with advanced hepatosplenic schistosomiasis might be the depressed immunity of such liver affected patients.

The use of immunological methods to detect minute amounts of antigen in body fluids (serum, urine) is an exciting diagnostic development and does not require the presence of viable organisms. An attempt has been made in this study to detect somatic Salmonella antigen in the sera of patients by ELISA, and revealed positive rates of 59.1% in group I, 28.6% in group II and 53.3% in group III. Abdel-Fattah et al⁽¹¹⁾, detected S. typhi somatic antigen in the sera of 57.1% of clinically suspected enteric cases, and a nearly similar result was reported by Taylor et al,⁽¹⁶⁾ who detected S. typhi antigen by ELISA in 62% of culture proven cases. Moderately lower results were obtained by Araj and Das Chugh⁽¹⁷⁾, who detected

Salmonella O-antigen by ELSA in (48%) of culture proven cases. On the other hand a high detection rate was reported by Appassakij et al,⁽²⁾ who detected *S. typhi* protein antigen in patients sera by ELISA in 83.87% of clinically suspected typhoid cases. However, this high detection rate is doubtful because the same authors have reported that cross-reaction can occur with high concentration of protein antigens obtained from some other related enteric bacteria.

The low rate by other authors, who referred it to the intracellular location of Salmonella, the low concentration of antigen in patient's blood due to clearance of the organisms by the reticuloendothelial system and separation of antigen-antibody complexes together with blood cells from serum.

It is quit clear from the results present in this study that antigen detection (ELISA) is a powerful indicator for Salmonella infection comparable to antibody detection (Widal test) in group I patients. This might issue the difference in reaction of immune system in such particular group of patients. Bilharzial infection seems to decrease the threshold of reactivity to other infections as clearly observed from Salmonella antibody detection in both group I and III. While the reverse was obviously found for antigen detection which was higher in group I compared to group III. It was striking to find the lower specificity of ELISA (Ag. detection) in group II patients compared to Widal test (Ab. detection) could be explained by the fact that this group of patients has no fever so it could be possible that the antigen level in patients serum is not so high compared to the antibody level.

Besides its good validity the ELISA established here also has advantages. First, it can be performed within the same day of sample collection. Also, the cost of ELISA was found to be lower than that of culture. Furthermore, the detection of bacterial antigens has the advantage over culture methods of being indicative of the presence of microorganisms viable to grow in vitro. (Haddock, 1985)¹⁸.

Thus, the results clearly indicate that the ELISA eliminates most of the shortcomings often encountered in the agglutination tests. Yet, antigen detection cannot completely replace culture

techniques since it does not predict antibiotic susceptibility patterns of the infecting organism (Fung and Tilton, 1985)¹⁹.

Thus, in view of what has been discussed, ELISA method for detection of Salmonella antigen could be a useful tool for the diagnosis of cases in conjunction with hemoculture and Widal test to obtain an early diagnosis of typhoid fever, particularly in patient of enteric fever associated with bilharzial hepatosplenomegaly.

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تقييم الاختبار الانزيمي المناعي (الاليزا) فى كشف مولدات المضادات (انتيجين) لميكروب السالمونيلا فى مرضى الكبد البلهارسى

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يهدف هذا البحث الى تقييم الاختبار الانزيمي المناعي (الاليزا) فى تشخيص الحمى المعوية
المصاحب لمرضى الكبد البلهارسى ومقارنته بالطرق التقليدية فى التشخيص مثل مزرعة الدم واختبار
فيدال. ولقد شمل الجزء العملى من هذا البحث ثلاث مجموعات :
المجموعة الاولى : ٢٢ حالة من مرضى الكبد البلهارسى مصحوبة بارتفاع فى درجة الحرارة.
المجموعة الثانية : ٢١ حالة من مرضى الكبد البلهارسى بدون ارتفاع فى درجة الحرارة.
المجموعة الثالثة : ١٥ مريضا وتم تشخيصهم اكلينيكيًا كحالات حمى معوية.

وبعد الفحص الاكلينيكي لتشخيص الحالات واختبارها اجريت الفحوص والاختبارات التالية :
فحص للبول والبراز للكشف عن بويضات البلهارسيا، وظائف للكبد، كشف بالموجات فوق الصوتية
، منظار شرجي، مزارع للدم ، اختبار التلزن (فيدال) ، الاختبار الانزيمي المناعي (الاليزا) لمعرفة
وجود انتيجين السالمونيلا فى مصل الدم.

وقد اسفرت نتائج هذا البحث عن الاتى : المجموعة الاولى : وجد ان هناك انتيجين
السالمونيلا فى ٥٩,١% من الحالات كما انه تم فصل الميكروب بطريقة مزرعة الدم فى ٩,١% من
الحالات بينما اعطى اختبار فيدال نتائج ايجابية فى ٤٠,٩% من الحالات. المجموعة الثانية : وجد ان
هناك انتيجين السالمونيلا فى ٢٨,٦% من الحالات وقد تم فصل الميكروب بطريقة مزرعة الدم فى
١٤,٣% من الحالات بينما اعطى اختبار فيدال نتائج ايجابية بنسبة ١٤,٣%. المجموعة الثالثة : وجد
ان اختبار الاليزا اعطى نتائج ايجابية بالنسبة لانتيجين السالمونيلا فى ٥٣,٣% من الحالات وقد تم
فصل الميكروب من ٤٠% من الحالات وقد اعطى اختبار فيدال نتيجة ايجابية بنسبة ٤٦,٧% .

ويستنتج من هذا البحث ان وجود بكتريا السالمونيلا قد يكون من المضاعفات الهامة والخطيرة
التي قد تصيب مرضى الكبد البلهارسى. وانه بمقارنة جميع النتائج ثبت ان اختبار الاليزا لاكتشاف
انتيجين السالمونيلا هو امثل الطرق للتشخيص النهائى السريع لحالات مرضى الحمى المعوية.