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Use of C-reactive protein in the evaluation of Widal test and Typhoid stripe test in the diagnosis of typhoid fever

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Abstract

Background: Typhoid fever is one of the systemic infection and responsible for high rate of mortality especially in poor hygiene provinces. The aim of this study was to evaluate the methods including C-reactive protein test, Widal test and Typhoid stripe test that used in the diagnosis of typhoid fever.

Material and Methods: Rapid direct Widal agglutination test and rapid typhoid stripe (immunochromatography) test were used to detect typhoid infection evaluate more accurate method in the diagnosis of typhoid fever. C-reactive protein detected by rapid slide agglutination test to measure its level in typhoid fever patients.

Results: The results of Widal test showed 29 (63%) of 46 sample while the results of typhoid stripe test showed 25 (54.3%) IgM positive and 2 (4.3%) IgG positive from 46 sample. The test of CRP accomplished in positive widal test and positive typhoid strip test samples, the results showed that 6 (20.6%) of 29 were positive and 6 (24%) of 25 were positive in widal and typhoid positive samples respectively.

Conclusion: Typhoid stripe test is more accurate and has low cross reactivity when compared with Widal test in the diagnosis of typhoid fever. Although, C-reactive protein test is non-specific but it is helpful in the characterization of acute inflammation and infection, so it is recommended to use in the detection of typhoid fever.

Key words: *Typhoid fever, Widal test, typhoid stripe test, CRP*

Introduction

The typhoid and paratyphoid fever stay important public health issues globally and major causes of morbidity in the developing world (1). Typhoid and paratyphoid fever are acute and sometimes serious febrile diseases caused by systemic infection with the *Salmonella enterica serotype typhi* and *paratyphi*, respectively (2). Clinical symptoms are similar but paratyphoid fever tends to have a more benign course of illness. Typhoid fever has a case-fatality rate of 10–30% without effective treatment. This rate is reduced to 1–4% in those receiving proper therapy (3).

It's an infrequent illness in developed countries that happens primarily in returning travelers, with occasional point-source epidemic. (4). The identified risk factors for disease include eating food prepared outside the home, (5,6) having a close contact or relative with recent typhoid fever (7), inadequate facilities for personal hygiene (8), drinking contaminated water, and recent use of antimicrobial drugs (6). S. *enterica serotype typhi* is a member of the family Enterobacteriaciae.

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The agent is serologically positive for lipopolysaccharide antigens (O9, O12), protein flagellar antigen (Hd), and polysaccharide capsular antigen (Vi). The Vi capsular antigen is largely restricted to *S. entericaserotype typhi,* although it is shared by some strains of *S. enterica* serotypes hirschfeldii (paratyphi C) and dublin, and *Citrobacter freu*ndii. A unique flagella type, Hj, is present in some *S. enterica serotype typhi* isolates from Indonesia (9). Moleculer methods like pulse-field gel electrophoresis, phage typing, and ribotyping have shown that endemic areas usually have many strains in circulation but that outbreaks are usually due to a restricted number of strains (10-12).

Salmonella organisms have an ability to survive and multiply within the mononuclear phagocytic cells (13). Organisms excreted in the bile either reinvade the intestinal wall or are excreted in the feces. Counts of bacteria in patients with acute typhoid fever indicate a median concentration of 1 bacterium per milliliter of blood and about 10 bacteria per milliliter of bone marrow (14,15). Although *S. enterica serotype typhi* produces a potent endotoxin, mortality from treated typhoid fever for patients at this stage is less than 1%. Studies have reported increased levels of circulating proinflammatory and antiinflammatory cytokines in patients with typhoid and a reduced capacity of whole blood to produce inflammatory cytokines in patients with severe disease (16).

C-reactive protein is one of the plasma proteins that termed acute phase proteins and show a dramatic increase in concentration in response to early 'alarm' mediators such as interleukin-l (IL-1) released as a result of bacterial infection. A major property of CRP is its ability to bind in a Ca-dependent fashion, as a pattern recognition molecule, to a number of microorganisms which contain phosphorylcholine in their membranes, the complex having the useful property of activating complement by the classical and not the alternative pathway. This results in the deposition of C3b on the surface of the microbe which thus becomes opsonized for adherence to phagocytes (17).

The potential role of CRP in the elimination of bacterial infection was experimented by transgenic mice that express high level of human CRP in serum against lethal infection by *Streptococcus pneumonia* (18). CRP transgenic mice also exhibit increased resistance to lethal infection against the Gram negative bacterium, Salmonella typhimurium (18).

The normal range of serum CRP in human is 0.3-1.7 mg/L and if the concentration raised above that indicate to the presence of inflammation (19). The secretion of CRP is begin rapidly and reach to the peak within 30-50 hours after stimulation and when the stimulus removed of disappeared the CRP fall and return to the normal level.

Elevation in serum CRP concentration include both infection with gram positive and gram negative bacteria in addition to fungal infection and there is limited knowledge about the change in CRP level in parasitic infection while many evidence related to never change in CRP level in viral infections. There is a slight elevation in the serum CRP concentration in the chronic infection and inflammation (18).

The aim of this study was to evaluate the traditional methods that used in the diagnosis of typhoid fever.

Methods and materials

Subject

Sixty four blood samples had been collected from patients suspected to have typhoid fever, the samples separated according to gender to 20 female and 26 male. All samples collected from Al-Hussein Teaching Hospital, Dhi-Qar Province, Iraq from December 2015 to March 2016.

Thirty two blood samples had been collected from apparently healthy persons as negative control.

Methods

Typhoid Rapid Test Cassette ABON® Company, China used in this study and the method done according to the manufacturer's leaflet. Widal test BIOLABO Company, France used and select rapid slide agglutination method as manufacturer's leaflet.

CRP (Biolabo Company, France) Qualitative agglutination test used in this study and the method done according to the manufacturer's procedure.

Statistical analysis: Statistical analysis estimated by used Fisher s exact test accomplished by SPSS software version 18 to identify the percentages of typhoid infections among suspected patients with two diagnostic methods and also measure the correlation coefficient between them and with CRP test.

Ethical Approval: This research underwent to the terms of ethical considerations and in accordance with the form prepared for this purpose by the Iraqi Ministry of Health,

also got the approval of the research by the Committee of ethical standards in the Faculty of Science, Thi-Qar University , one of the colleges affiliated to the Ministry of Higher Education and Scientific Research, Iraq.

Results

The results of Widal test showed 29 (63%) of 46 sample were positive and 17 (37%) were negative, for the same manner, 24 (75%) of 32 control samples gave positive results for widal test although they were not exposed any typhoid fever symptoms and just 8 (25%) of 32 were negative (Table 1).

Table 1. The number and percentage of Widal test positive result among typhoid fever patients and controls.

Samples		Widal Test		Total
		Positive	Negative	
Patient:	**Count**	29	17	46
	%	63%	37%	100%
Control:	**Count**	24	8	32
	%	75%	25%	100%
Total:	**Count**	53	25	78
	%	68%	32%	100%

Table 2. The number and percentage of Typhoid Strip test IgM positive result among typhoid fever patients and controls.

Samples		Typhoid Strip Test IgM		Total
		Positive	Negative	
Patient:	**Count**	25	21	46
	%	54.3%	45.7%	100%
Control:	**Count**	4	28	32
	%	12.5%	87.5%	100%
Total:	**Count**	29	49	78
	%	37.1%	62.9%	100%

Typhoid strip tests (immunochromatography) gave more accuracy results and classified the patients into acute and chronic infection. The results showed 25 (54.3%) IgM positive and 2 (4.3%) IgG positive from 46 sample. According to control, the results showed 4 (12.5%) IgM positive and 0 (0%) IgG positive from 32 control in typhoid strip test (Table 2 and Table 3).

Table 3. The number and percentage of Typhoid Strip test IgG positive result among typhoid fever patients and controls.

Samples		Typhoid Strip Test IgG		Total
		Positive	Negative	
Patient:	**Count**	2	44	46
	%	4.3%	95.7%	100%
Control:	**Count**	0	32	32
	%	0%	100%	100%
Total:	**Count**	2	76	78
	%	2.5%	97.5%	100%

The test of CRP accomplished in positive widal test and positive typhoid strip test samples, the results showed that 6 (20.6%) of 29 were positive and 6 (24%) of 25 were positive in widal and typhoid positive samples respectively (Table 4).

The results of correlation coefficient between CRP test with widal test and typhoid strip IgM test showed significant correlation of 0.297 and 0.355 respectively, and 0.058 with typhoid strip IgG test (table 5). Significant differences ($P<0.01$) had been observed in the detection of CRP level in both Widal test positive and typhoid strip IgM positive samples when compared with negative controls.

Table 4. The number and percentage of CRP test positive result among Widal test and typhoid strip test positive samples and controls.

Samples	CRP test				Total of CRP Positive
	Widal Test		Typhoid Strip Test		
	Positive	**Negative**	**Positive**	**Negative**	
Patient					
n	6	23	6	19	12
%	20.6%	79.4%	24%	76%	22.3%
Control					
n	0	32	0	32	0
%	0%	100%	0%	100%	0%
Total					
n	6	55	6	51	12
%	9.8%	90.2%	10.5%	89.5%	10%

Discussion

The results of current study indicate to the high accuracy of typhoid strip test in the diagnosis of typhoid fever rather than Widal test and this was evidenced by the low positivity among control group those were not complain from typhoid fever symptoms.

Other studies (20) indicate to the presence of antigen mimicry between Salmonella typhi and other pathogens and this tested by negative culture of S. typhi in those gave positive widal test. So that the result of the current study indicate the presence of cross reactivity especially in control group.

Poorly performance of rapid slide agglutination of Widal test had been presented by Danu et al (21) study which explain it had very poor specificity even though it was performed under optimal conditions in a national reference laboratory. This poor performance was further compounded by substantial inter-test variability, which suggests that in a field situation results would not be comparable between sites, and this observation also presented by the current study.

The CRP response is very non-specific and can never be used as a single diagnostic tool, however it is very helpful in several disease states. Its application in infectious diseases is unquestionable (22). Changes in CRP level are very helpful in diagnosis as well as in monitoring response to therapy, as CRP levels are only determined by the rate of synthesis. Contrarily, other acute phase phenomena such as leukocytosis and fever are dependent on complex mechanisms involving several mediators. Therefore, these markers are not reliable markers of sepsis (22).

The observation of current study showed significant correlation between increased level of CRP and positive results of both Widal test and typhoid stripe IgM test and this result is similar to that observed by study of (23). It has been observed that the levels of C-reactive protein (CRP) become high in patients of typhoid fever more aggressively. A strong correlation was found between augmentations in C - reactive protein due to rise in immunoglobulin M (24).

This result similar to the result of other studies showed high association between positive culture of Salmonella typhi and CRP test (25). These give an evidence to use the CRP test in the diagnosis of acute infection of typhoid fever and depend more on the typhoid strip test in the diagnosis.

Conclusion

Rapid slide agglutination of Widal test is non-specific for diagnosis of typhoid fever because the cross reactivity that observed when gave positive results in healthy control persons. Typhoid stripe test that depend on the lateral flow or immunochromatography technique is more precise in related to Widal test and classify the typhoid infection into acute and chronic according to the type of antibody (IgM or IgG) that detected in the patient's serum. CRP could be used as assistance diagnostic tool in the detection of typhoid fever because its characteristic of rising level in the acute inflammation and infection.

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