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Original article

Circulating interleukin-6 and tumor necrosis factor receptor-1 predict resistance to therapy of typhoidal salmonellosis

***Background**: Typhoid fever, a food-borne disease caused by salmonella species, is a worldwide prevalent disease. In endemic areas, children are at highest risk owing to weaning from passively acquired maternal antibody and lack of acquired immunity. Several studies have been done to clarify the pathogenesis and underlying immune aspects of typhoid fever. **Objective**: Study the changes of some proinflammatory cytokines in plasma of children with typhoid fever. **Study design**: Thirty consecutive children admitted to Zagazig Fever Hospital with proven diagnosis of typhoid fever were included in the study. They were 20 males and 10 females, of ages ranging from 3 to 13 years. In addition, 10 age and sex matched healthy children served as a control group. A verbal consent was obtained from parent(s) of each child before inclusion to the study. All children were subjected to history taking, clinical examination, and routine investigations (CBC, ESR, CRP, Widal test and stool culture), as well as determination of serum interleukin-6 (IL-6) and tumor necrosis factor receptor-1 (TNF-R1), before and 5 days after start of treatment (for patients). **Results**: Twenty patients (66.7%) were responsive to therapy and 10 patients (33.3%) were resistant. Toxic look, constipation, high fever, splenomegaly, increased CRP and ESR were significantly presented in patients who displayed resistance to drug therapy. Both IL-6 and TNF-R1 plasma levels were significantly higher in patients than in control children, and in resistant cases than in responsive cases (before and 5 days after treatment). ESR and S. typhi H agglutination titre correlated significantly with plasma levels of IL-6 and TNF-R1, whereas S. typhi O agglutination titre and total leucocytic count did not. **Conclusion:** patients with typhoid fever resistant to combined therapy with chloramphenicol and co-trimoxazole have higher plasma levels of IL-6 and TNF-R1. Toxic look, constipation and splenomegaly may be considered as indicators of drug resistance.*

Key words: salmonellosis - resistance to therapy – cytokines.

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INTRODUCTION

Typhoid fever, encountered in all parts of the world, is nowadays primarily found in countries where sanitary conditions are poor. It is considered to be one of the most important and under-reported diseases in the developing world. In some areas, it has been estimated that typhoid fever is responsible for 2 to 5% of all deaths. Hospital case fatalities are still high (1 to 30%), in many parts of the developing world[1]. In the US, the incidence was 400 cases/year, mostly among travelers[2]. In Egypt, Crump et al.[3] estimated the incidence of typhoid fever to be 13/100000 persons.

S. typhi, the etiologic organism of typhoid fever, is similar to other Salmonellae in that it is a gram-negative, flagellated, non-capsulated, non-sporulating, facultative anaerobic bacillus. It has a somatic (O) antigen (oligosaccharide), flagellar (H) antigen (protein), and an envelop (K) antigen (polysaccharide); and has a lipopolysaccharide macro-molecular complex called endotoxin that forms the outer portion of the cell wall[4].

Cellular immune response, a major characteristic of typhoid fever, entails activation of macrophages. Phagocytosis is a major host defense mechanism, and substances released from macrophages, including cytokines, probably have a significant role in the pathogenesis of the disease[5]. So this work aimed to study the changes in the levels of IL-6 and TNF-R1, as well as the effect of these changes on the clinical course of children suffering from typhoid fever.

METHODS

The study included 30 children (20 males and 10 females), of ages ranging from 3 to 13 years (X ± SD: 8±4.7 years), who were admitted to Zagazig Fever Hospital, during the year 2008. The isolate organism confirmed to *Salmonella* by stool culture on selenite broth[6].

Ethical approval was obtained from the local research ethics committee and parents of all subjects gave an informed written consent prior to the study.

All patients were given oral chloramphenicol (in a dose of 100 mg/kg/day) divided every 6 hours and oral cotrimoxazole (in a dose of 48-60 mg daily) in 2 divided doses. According to the response to therapy, at the 5th day[7], patients were divided into 2 groups:

Group I (Responsive): Twenty patients (12 males and 8 females), of mean age 8±5 years who showed clinical improvement with treatment.

Group II (Resistant): Ten patients (6 males and 4 females), of mean age 7.75±4.2 years, who did not show improvement after 5 days of therapy.

Ten healthy children (6 males and 4 females) of mean age 8.1±5.3 years were studied as control group.

All children were subjected to the following:

1. Clinical history taking including time of onset and character of fever, chills, headache, loss of appetite, constipation or diarrhea and abdominal pain
2. Physical examination including diurnal body temperature measurement, heart rate, splenomegaly and skin rash.
3. Routine laboratory investigations, including complete blood count (CBC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)[8]. The diagnosis of typhoid fever was confirmed by Widal test using Murex-stained *Salmonella* suspension (Dartford, UK)[9], as well as isolation of *S. typhi* after stool culture, according to the method described by Old[10].
4. Measurement of plasma IL-6 and TNF-R1 on admission and on day 5 after treatment. IL-6 measurement was done using a commercially available ELISA kit protocol (Cell Science, Inc. Catalog No. 850.030.096). The cut off level of IL-6 was 0.99 pg/ml. TNF-R1 measurement was done using PREDICTA TNF-R1[11]. The cut off level of TNF-R1 was 64.0 pg/ml.

Statistical analysis[12]

Data were presented as mean ± standard deviation (X±SD) or percentage (%). The means of two groups were compared using student "t" test. Linear correlation and regression were used to test the correlation between the measured cytokines (IL-6 and TNF-R1) and some laboratory tests in all patients. Odds ratio was used to quantify the risk. Cut off values were calculated as mean ± 2SD of control.

Data were carried out with the statistical package for Social Sciences (SPPS), version 10 software. P-values less than 0.05 were considered significant.

RESULTS

Analysis of clinical and laboratory characteristics revealed that toxic look, constipation, high fever, splenomegaly, and increased CRP and ESR were significantly more prevalent in children resistant to therapy of typhoid fever than in responsive children (Table 1).

Table 2 shows that both IL-6 and TNF-R1 plasma levels were significantly higher in patients than in controls and in resistant cases than in responsive cases both before and 5 days after treatment.

Significantly higher IL-6 was noticed in patients after treatment, both the responsive and the resistant, than in controls. The same applies to TNF-R1 (Table 3).

IL-6 levels correlated significantly with ESR values but not with *S. typhi* O and H titres nor TLC. On the other hand TNF-R1 correlated significantly with *S. typhi* H agglutination titres and ESR, with nonsignificant correlation with *S. typhi* O agglutination titres and TLC (Table 4).

Table 1. Clinical and laboratory characteristics in responsive and resistant patients.

Characters	Responsive Patients (*n* = 20)	Resistant Patients (*n* = 10)	OR (95% CI)	*P*
Symptoms (*n* and %)				
Abdominal pain and anorexia	18 (90)	8 (80)	0.44 (0.03-5.58)	0.58
Headache and malaise	11 (55)	4 (40)	0.55 (0.09-3.24)	0.43
Chills	8 (40)	2 (20)	0.38 (0.04-2.82)	0.41
Diarrhea	6 (30)	2 (20)	0.58 (0.06-4.62)	0.68
Constipation	4 (20)	8 (80)	16 (1.87-180.33)	0.004*
Toxic look	7 (35)	8 (80)	7.43 (0.98-69.99)	0.02*
Signs (X±SD)				
Temperature (°C)	38.6 ± 0.4	40 ± 0.6		< 0.001**
Liver span (cm)	8 ± 2	7.5 ± 1.6		0.49
Spleen below costal margin (cm)	1 ± 0.5	3 ± 1.		0.22
Laboratory data (X±SD)				
TLC ($x10^3/mm^3$)	5.2 ± 1.4	6.6 ± 2.6		0.23
ESR (mm/h)	60 ± 8.7	132.5 ± 15.1		0.02*
CRP (mg/L)	27.5 ± 9.1	55 ± 6.8		<0.001**

ESR: erythrocyte sedimentation rate, OR: odds ratio, CI: Confidence Interval, TLC: total leukocyte count, CRP: C-reactive protein, *: significant, **: Highly significant.

Table 2. Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor receptor-1 (TNF-R1) in all studied groups.

Variable	X ± SD	*P*
IL-6 (pg/ml)		
Control (*n* = 10)	0.57 ± 0.21	0.01*
All patients before treatment (*n* = 30)	6.23 ± 4.71	
Before treatment		
Responsive patients (*n* = 20)	5.05 ± 2.32	0.039*
Resistant patients (*n* = 10)	7.38 ± 4.10	
After treatment		
Responsive patients (*n* = 20)	2.22 ± 0.81	0.035*
Resistant patients (*n* = 10)	3.74 ± 0.73	
TNF-R1 (pg/ml)		
Control (*n* = 10)	35.66 ± 14.21	0.001*
All patients before treatment (*n* = 30)	131.50 ± 18.34	
Before treatment		
Responsive patients (*n* = 20)	81.7 ± 14.22	0.001*
Resistant patients (*n* = 10)	165.68 ± 17.35	
After treatment		
Responsive patients (*n* = 20)	57.92 ± 11.06	0.01*
Resistant patients (*n* = 10)	101.38 ± 16.03	

* : significant, pg: picogram.

Table 3. Interleukin-6 (IL-6) and tumor necrosis factor receptor-1 (TNF-R1) in patients after treatment compared to control group.

Variable	Control (*n*=10)	Patients after treatment (*n*=30)		*F*	*p*
		Responsive (*n*=20)	Resistant (*n*=10)		
IL-6 (pg/ml)	0.57±0.21*	2.22 ±0.81	3.74±0.73	52.69	<0.001**
TNF-R1 (pg/ml)	35.66±14.21*	57.92±11.06	101.38±16.03	66.12	<0.001**

*: $p < 0.001$ when controls were compared with responsive or resistant patients.
**: highly significant

Table 4. Correlation between the measured cytokines (IL-6 and TNF-R1) and some laboratory investigations in study patients.

Variable	IL-6	TNF-R1
	r	*r*
***S. typhi* O agglutination titre**	0.033	0.116
***S. typhi* H agglutination titre**	0.023	0.98*
Total leucocytic count	0.022	-0.101
Erythrocyte sedimentation rate	0.579*	0.643*

*: significant

DISCUSSION

In this study we classified our patients into 2 groups according to the clinical response to a combination of chloramphenicol and co-trimoxazole. Responders were 20 cases (66.7%) and resistant cases were 10 (33.3%). This response rate is similar to that reported by other studies[13,14]. Furthermore, Tohme et al.[14] added that in spite of in vitro resistance, chloramphenicol and co-trimoxazole remain the first choice treatment of enteric fever as they usually achieve clinical cure in the majority of typhoid patients.

In this study, toxic look, constipation, high fever, marked splenomegaly, and raised CRP significantly characterized resistant cases. The variation in the frequency of clinical features may be explained by variation in age, as infants and young children, for example, develop diarrhea rather than constipation[15]. Also this may be due to variations in endotoxin production, local inflammatory and/or host immune reactions between responsive and resistant cases[16].

In this study, resistant cases had a significantly higher mean ESR than patients who responded to drug therapy. Wyant et al.[17] attributed this increase to increased IL-6, and considered it as one of the major physiological mediators of acute phase reaction. They confirmed this observation with significant positive correlations of IL-6 with fever, ESR and CRP.

In our series, patients had significantly higher IL-6 levels, before treatment compared to controls. This increased level of IL-6 may be due to enhanced IL-6 gene expression in human monocytes stimulated by *S. typhi* proteins[18]. In addition bacterial antigen is usually recognized by intestinal epithelial cells via toll-like receptors to initiate the cellular immune responses, where elevated IL-6 is one of its manifestations[19]. Resistant patients had significantly higher levels of IL-6, before treatment, compared with responders, indicating a prognostic value of IL-6. This is in agreement with Bhutta et al.[20].

Five days after treatment, the decline of IL-6 was significant in both responsive and resistant patients. This finding clarifies the inflammatory role of IL-6 and that it is one of the mediators responsible for persistence of inflammatory manifestations, especially in the resistant patients[21].

In this study, there was significantly higher TNF-R1 in all patients compared with controls. This was more obvious in resistant cases compared with responsive cases both before and 5 days after treatment. This agrees with Beutler et al.[22] who reported higher levels of TNF-R1, in resistant typhoid patients, associated with prolonged fever or relapse after treatment with chloramphenicol, when compared with responsive patients. They concluded that such higher levels of TNF-R1 may be capable of binding TNF and thus enhancing its harmful effects. It is to be noted that the levels of TNF-R1 declined significantly after 5 days of treatment. This agrees with the findings of Wyant et al.[17] who found that stimulation with *Salmonella typhi* flagella has induced rapid de novo synthesis of TNF-R1, during acute typhoid illness which decreased with convalescence.

Upon correlation of studied cytokines and some laboratory data, we found significant positive correlation of IL-6 with ESR (r = 0.579). This result was in agreement with Zherebtsova et al.[23] who found medium positive relationship between the level of IL-6 and changes in blood including ESR. Also our study showed significant positive correlations of TNF-R1 with *S. typhi* H agglutination titre and with ESR which may reflect the positive activities of these cytokines[24].

In conclusion, the studied children with typhoid fever who were resistant to treatment with chloramphenicol and co-trimoxazole had higher levels of IL-6 and TNF-R1 than those who were responsive. Toxic look, constipation and splenomegaly may be considered as indicators of drug resistance. IL-6 and TNF-R1 have a role in the pathogenesis of typhoid fever and we recommend further studies to define the exact role of immunomodulation in treating resistant cases.

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