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Original Contribution

DYNAMICS OF SERUM PRO-INFLAMMATORY CYTOKINES IN PATIENTS WITH SALMONELLA INFECTION

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ABSTRACT

A prospective study on serum levels of several pro-inflammatory cytokines was carried out in patients with salmonellosis. The aim of the study was to establish a correlation between cytokine levels on one hand and the severity of disease on the other. The study included 37 patients with culture confirmed gastrointestinal Salmonella infection. They were hospitalised at the Clinics of Infectious Diseases, St. George University Hospital, Plovdiv, Bulgaria. Patients were aged between 19 and 57 years. 12 of them had mild, 14 moderate and 11 severe forms of the infection. 25 age-matched subjects served as controls. Serum levels of IFN-γ, TNF-α and IL-1β were determined, using ELISA (Biosource, Belgium), during the acute (day 2-4) and convalescence (day 10-12) disease periods. Levels were presented as X±SD. IFN-γ serum levels were significantly elevated in the acute (1.97±1.39 UI) and convalescence stage (1.04±0.95 UI) when compared with controls (0.175 ±0.12 UI). Similar dynamics were established in TNF-α serum concentration – 68.12±38.22 and 50.68±37.42 pg/ml in patients with salmonellosis (acute and convalescent stage, respectively) and 10.50±6.38 pg/ml in healthy controls. IFN-γ and TNF-α serum levels were significantly higher in patients with severe clinical forms of infection. Serum levels of IL-1β were significantly elevated during the acute stage of disease (165±91.08 pg/ml) and during convalescence (94±60.75 pg/ml) when compared to controls (15±12 pg/ml) but without significant difference among patients with various severity of the disease. IFN-γ and TNF-α serum levels have additional diagnostic value in determining course severity of salmonellosis.

Key words: cytokines, salmonellosis, IFN-γ , TNF-α, IL-1β

INTRODUCTION

Salmonelloses are important, not only as a challenge to public health, but also as a model for studying the fundamental pathogenic mechanisms of bacterial infections (1, 2) The complex interactions between *Salmonella* bacteria and the infected host are not completely understood and are still subjects of intense investigations (1, 3, 4, 5).

Cytokines as intercellular molecules mediate the effect of main pathogenic factors of *Salmonella* and are responsible, to a great extent, for the pathological processes in *Salmonella* infection. Cytokines are antigen-non-specific signal proteins, produced by a variety of cell types in response to various stimuli induced by bacteria and their products, viruses, parasites, nucleic acids, other cytokines, etc (6, 7). They participate in inflammatory and immune reactions of the host, regulating proliferation, differentiation, migration and activation of T and B cells, macrophages, polymorphonuclear cells and endothelial vascular cells as well as humoral defence factors (8, 9, 10). Cytokines may be classified as pro-inflammatory (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8) and anti-inflammatory (IL-14, IL-10, IL-13). The pro-inflammatory (alarm) cytokines induce febrile reaction, stimulate hepatocytes to synthesise acute phase proteins and hypophysis to secrete stress hormones. Inducing proliferation and haemotaxis of polymorphonuclear cells to the site of inflammation, cytokines cause vascular dilatation, increase permeability and adherence of endothelial cells in the damaged site (3, 6, 9, 11, 12).

The main activator of cytokine induction in

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Salmonella infection is the endotoxin with pronounced pluripotent ability to interact in non-cytotoxic manner with host cells (1, 13, 14, 15). *Salmonella* porins and flagella also participate in induction of cytokine synthesis (16, 17, 18).

Studies on pro-inflammatory cytokine levels in patients with salmonelloses are still limited. The type and dynamics of cytokine response in humans are still not completely understood. Consequently the present investigation focuses on how cytokines participate in pathogenesis and immunogenesis of the disease. In our previous study we demonstrated that elevated serum concentrations of several cytokines - IFN-γ, TNF-α and IL-12, correlated with early bacterial clearance in patients with gastro-enteric *Salmonella* infection, thus suggesting that these cytokines probably participate in protective immunity of the disease (19). We have also found that serum concentrations of cytokines correlated with some laboratory parameters in patients with salmonellosis (20).

This study extends our investigations on human salmonellosis and is aimed at establishing the serum levels of pro-inflammatory cytokines, IFN-γ, TNF-α and IL-1, in patients with salmonellosis, and the relationship of these cytokines with disease severity.

MATERIALS AND METHODS

Patients. The study enrolled 37 patients with culture proven for salmonellosis, treated at St. George University Hospital in Plovdiv, Bulgaria. Age range among patients was 18-57 years. Twelve of them presented with mild infection; moderate disease was registered in 14 patients and severe in 11. *Salmonella enterica* serotype *Enteritidis* was the pathogenic agent in 81% of the cases, while *Salmonella enterica* serotype *Typhimurium* in 13.51%. Other *Salmonella* serotypes (mainly group C) were isolated in 5.49% of the patients. Control group consisted of 25 healthy age-matched subjects.

Table 1. *Criteria for severity evaluation in slamonellosis*

Clinical forms *symptoms syndroms*	**Mild**	**Moderate**	**Severe**
Fiver degree duration	up to 38°C 1-3 days	38°C – 39°C up to 5 days	over 39°C over 5 days
Vomiting intensity *duration*	1-3/24h 1 day	4-8/24h 2-4 days	over 8/24h over 4 days
Diarrhoea intensity *duration*	5-6/24h 5 days	6-10/24h 6-10 days	over 10/24h over 10 days
Intestinal crams	negligible	moderate to severe	severe and prolonged
dehydration	No or 1st degree	1st to 2nd degree	2nd to 3rd degree
Central nervous system symptoms	No or mild headache	severe headache	hyperesthesy, seizures, mental obtundation
Cardio-vascular disturbances: tachycardia hypotonia	100 min^{-1} up to 100 mm	100-120min^{-1} up to 80 mm	over 120 min^{-1} up to 60 mm
Renal disorders	minimal oliguria, high urine gravity	oliguria, elevated urea, normal or elevated creatinine levels	oligo-anuria, elevated urea and creatinine levels

Clinical criteria and definitions. *Acute stage of the disease* was defined as the period of main clinical symptoms presenting fever, diarrhoea and general weakness, which usually ranged from 4 to 6 days. *Convalescence* was characterised as the period when main symptoms faded away and the condition of patients improved. This generally happens between the 8th and 15th day from disease onset. However, consensus criteria for *disease severity* of gastro-intestinal salmonelloses do not exist. Based on clinical analysis of more than 1000 patients with salmonellosis, the authors propose parameters for assessment of disease severity according to the degree of manifestation of general toxic symptoms, intensity and duration of gastro-intestinal signs, dehydration and complications (5). These are presented on **Table 1** as mild, moderate and severe clinical variants of salmonellosis.

Blood samples were collected from patients repeatedly – during the acute stage of disease and during convalescence. Serum levels of IFN-γ, TNF-α and IL-1β were measured by enzyme immunosorbent assay (ELISA). All kits were commercially available and purchased from *Biosource Medgenics Diagnostics Fleurus*, Belgium. The levels of serum cytokines were analysed

according to the manufacturer's instructions. The detection limits of the ELISA kits for IFN-γ, TNF-α and IL-1β were: 0.03 IU/ml, CV 7.7-8.1%; 3 pg/ml, CV 8-9%; 2pg/ml, CV 1.5-2.2% respectively. Data were processed using Student's t- test.

RESULTS

Serum IFN-γ, TNF-α, and IL-1β levels (X±SEM) were markedly elevated during the acute disease stage and during convalescence and showed a tendency to normalise in the course of disease (**Figure 1**).

Comparative assessment of cytokine levels among patients presenting with different severity of salmonellosis demonstrated that IFN-γ and TNF-α levels were significantly higher in patients with severe disease course (**Figure 2**). Serum IL-1β did not correlate markedly with disease severity.

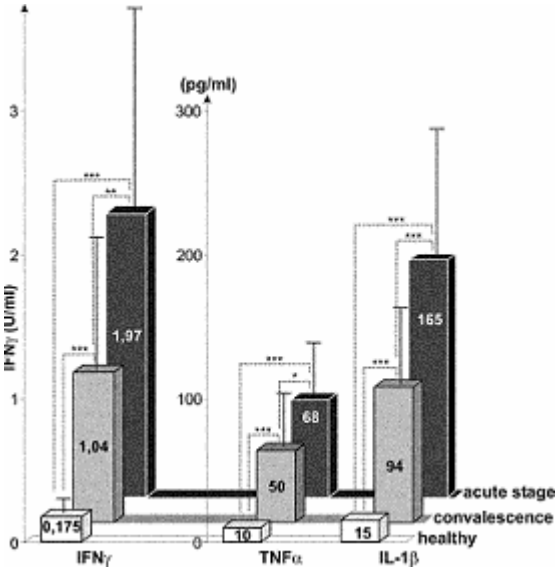

Figure 1**. Serum levels of IFN-γ, TNF-α and IL-1β (X±SD) during the course of salmonellosis. Significant differences are indicated as one (* p<0.05), two (** p<0.01) or three (p<0,001) asterisks.*

DISCUSSION

Data concerning levels of pro-inflammatory cytokines in *Salmonella* infection are mainly from studies on animal (mouse) models. Experimental *Salmonella dublin* infection in mice induced sharp increase of pro-inflammatory cytokines including IL-1 and TNF (21). In contrast, Jotwani found slight elevation of these cytokines in *Salmonella* sepsis (22).

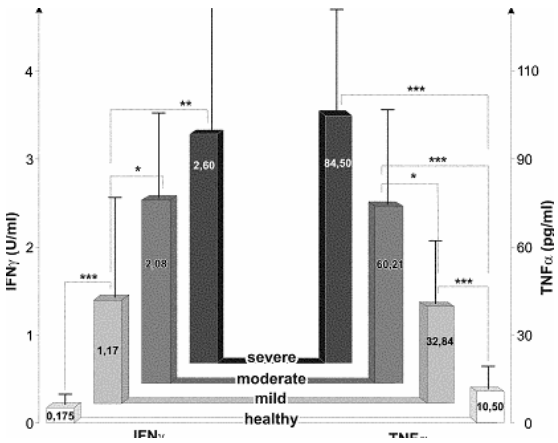

Figure 2.** Serum levels of IFN-γ and TNF-α (X±SD) during acute stage of salmonellosis in patients with various severity of the disease – mild, moderate and severe course. Significant differences are indicated as one (* p<0.05), two (** p<0.01) or three (p<0,001) asterisks.*

The results of our study demonstrate elevated serum levels of the cytokines involved in acute inflammatory reactions in patients with salmonellosis, peaked at the disease climax. A possible explanation of this pattern of kinetics is the large amount of inductors for cytokine synthesis – *Salmonella* endotoxin, porins and flagellin, which have their maximum at this period (16, 23).

Surprisingly, no correlation between serum IL-1β level and disease severity was established in our study. It is known that this cytokine plays a key role in mediating inflammatory reactions and tissue alterations (21,24). It has a broad spectrum of action – pro-inflammatory activity and also induction of defence reactions to the infection site (21). It is likely that the absence of correlation is attributable to IL-1β-induced secretion of its physiologic inhibitor - IL-1ra (24). This feedback mechanism may be protective against increased IL-1β level.

Experimental studies showed that *Salmonella spp*. induce early production of TNF-α (17, 25). Its sharp increase leads to pathological reactions and tissue alteration of gut epithelium and diarrhoea of invasive inflammatory type (16, 25, 26). According to the results of this study there is a correlation between serum TNF-α levels and disease severity. It might be considered that elevated TNF-α levels are responsible for the more severe course of *Salmonella* infection in humans. Similar correlation has been established in patients with toxic shock, typhoid fever and shigellosis (16, 27, 28, 29).

Several experimental investigations on *Salmonella* infection have found Th1 type

immune response, for which IFN-γ is indicative (22, 30). According to some authors, this cytokine together with TNF-α, contributes to the control of bacterial replication during the earliest stages of *Salmonella* infection (11) and of extreme inflammation by inducing IL-1ra production (31). The protective effect of IFN-γ has been demonstrated in patients with genetic deficiency for its production (in the IFN-γ gene or IFN-γ receptor). These patients are typically prone to *Salmonella* infections (32).

CONCLUSIONS

Serum levels of the pro-inflammatory cytokines IFN-γ, TNFα and IL-1β are elevated in patients with *Salmonella* infections. IFN-γ and TNF-α levels are increased markedly in the acute disease stage and in severe clinical forms. They have additional diagnostic value for disease severity in gastro-intestinal salmonellosis.

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