CORRELATION BETWEEN SERUM LEVELS OF INTERLEUKIN-1\(\beta\), INTERLEUKIN-1RA, INTERLEUKIN-6, INTERLEUKIN-10, INTERLEUKIN 12, TUMOR NECROSIS FACTOR-\(\alpha\) AND INTERFERON-\(\gamma\) WITH SOME CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH SALMONELLOSIS

M.S. Stoycheva\(^1\), M.A. Murdjeva\(^2\)
University Hospital “St. George”, Department of Infectious Diseases, Plovdiv, Bulgaria\(^1\)
University Hospital “St. George”, Department of Microbiology and Immunology, Plovdiv, Bulgaria\(^2\)

ABSTRACT

Salmonella bacteria are the major cause of food-borne infectious diarrhea. There is great interest in understanding the pathogenesis and immunogenesis of Salmonella infection. Cytokines are key communicator molecules between host cells and the enteric pathogen Salmonella. Serum levels of IL-1, IL-1ra, IL-6, IL-10, IL-12, TNF-\(\alpha\) and IFN-\(\gamma\) were studied in a prospective controlled study including 36 patients at the acute stage of gastrointestinal salmonellosis. Correlation between them was investigated, as well as between cytokines, body temperature and serum levels of Na\(^+\), K\(^+\) and Cl\(^-\). The lowest correlation was found between TNF-\(\alpha\) and IL-1ra. A significant and directly proportional correlation was established between IL-12 with IFN-\(\gamma\) and TNF-\(\alpha\), as well as in TNF-\(\alpha\)/IL-1\(\beta\) and IL-1\(\beta\)/IL-1ra. The increased body temperature correlated significantly with IL-1\(\beta\), TNF-\(\alpha\) and IL-6. Significant but converse was the correlation between Na\(^+\) with IL-1\(\beta\) and IL-6, as well as between body temperature with Na\(^+\), K\(^+\) and Cl\(^-\). Our data imply evidence for the complicated network in which cytokines act with multiple potential synergistic and antagonistic effects between them participating in the main pathogenetic processes – intoxication and loss of fluids and electrolytes.

Introduction

Cytokines are involved in initiation, amplifying, perpetuating and resolving the inflammatory and immune responses. They are key mediators for tissue damage and play an important role in many systemic disease manifestations (25).

Abbreviation: Interleukin-1\(\beta\) (IL-1\(\beta\)), Interleukin-1ra (IL-1ra), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Interleukin-12 (IL-12), Tumor Necrosis Factor – \(\alpha\) (TNF-\(\alpha\)), Interferon-\(\gamma\) (IFN-\(\gamma\))

Cytokines are classified as pro-inflammatory (TNF-\(\alpha\), IL-1, IL-2, IL-6, IL-8, IFN-\(\gamma\)) and anti-inflammatory (IL-4, IL-10, IL-13). The pro-inflammatory ones are “alarm” cytokines, inducing vascular dilatation with increased permeability and enhanced adherent ability of endothelial cells. These cytokines enable proliferation and hemotaxis of polymorphonuclear leukocytes to the place of injury and lead to further tissue damage (10,21,28,30). They also stimulate the pituitary gland for releasing
stress hormones and the hepatocytes for acute-phase protein synthesis (28). The acute-phase response includes as well mechanisms for control upon the inflammatory reaction – secretion of anti-inflammatory cytokines, receptor antagonists and cortisol.

The insufficient cytokine production or the inability for adequate response to them may lead to immune suppression, but their excessive increase – to shock and autoimmune disorders (12,16,25,28). Recently the pathological events, mediated by cytokines, have been connected mainly with imbalance in production of pro-and anti-inflammatory ones. The ration between them is proportional to the influence for their production (15,31,38). When the cytokines act mainly locally in the inflammation site (in auto- or paracrine manner), the favorable effects for the body dominate. They are directed to stimulation of defense mechanisms. On the contrary, extremely elevated serum concentrations result in prevalence of their destructive effects (4,21). According to others, neither peak, nor mean serum levels are decisive for the degree of damage but the time duration for cytokine increase (24).

In Salmonella infection the main activator of cytokine induction is the lipopolysaccharide (endotoxin) with marked multipotent ability for noncytotoxic interaction with host cells and tissues and their activation (2, 20, 25, 35). Salmonella porines and flagellins are other activators too (5,13,37).

Some experimental works report that Salmonella spp. as intracellular pathogens induce Th1 related inflammatory response (14, 23, 31) and the tissue alteration is preceded by release of pro-inflammatory cytokines (22). In patients with salmonellosis, the type of cytokine response and the importance of cytokine for the course and outcome of the disease, are still unclear. Our recent investigations showed that IL-1β and IL-ra serum levels were increased in these patients during acute and convalescent stage of salmonellosis (29).

The aim of this study was to investigate the correlation between serum levels of IL-1β, IL-1ra, IL-6, IL-10, IL-12, TNF-α and IFN-γ; the febrile reaction and serum levels of Na⁺, K⁺ and Cl⁻ in patients during acute stage of salmonellosis.

Materials and Methods

The study included 36 patients (aged 19-56 years) with gastrointestinal salmonellosis. They were treated at the Clinics of Infectious Diseases, University Hospital – Plovdiv. All patients had culture confirmed diagnosis. S. enteritidis was the etiological agent in 81% of the cases, S. typhimurium – in 13.5% and other Salmonella serotypes - in 5.5%.

Investigations were performed during acute disease stage. Blood samples for electrolytes and cytokines were collected upon admission and before treatment initiation. Serum levels of Na⁺, K⁺ and Cl⁻ were detected in arterialized capillary blood with an automated analyzer (288 Blood-Gas System, Ciba Corning). The analysis of electrolytes was performed immediately after the blood collection.

Cytokine levels were measured from sera using ELISA kits (BioSource, Medgenix Diagnostics, Fleurus, Belgium) according to manufacturer’s instructions and as described briefly before (29). Serum concentrations were determined for all cytokines in one experiment. The sera were frozen at –70 ºC for no longer than 6 months and then thawed at the day of testing.

The body temperature of all patients was appreciated as the main component of toxic infectious syndrome, and the electrolyte disorders as the most important markers for gastro-enteric dysfunction in salmonellosis.

Descriptive statistics and correlation analysis using Microsoft Excel 2000 were applied. The degree of correlation was assumed on the basis of Pearson coefficient (rxy). The correlation was significant at 0.01 level (2-tailed).
Results and Discussion

Correlation between serum levels of tested cytokines. A high and directly proportional correlation was observed between TNF-α and IL-ra. This confirms the data by others (1,32), who suggested that TNF-α together with IL-1 and the Salmonella endotoxin are inducers for IL-ra production. Probably this is a manner to control the inflammatory reaction by blocking the inflammatory effect of IL-1, which physiological inhibitor is IL-1ra.

Our findings showed that a significant correlation existed between serum levels of IL-12 with TNF-α and IFN-γ. This is in concordance with the data of Mizuno J et al (21) in patients with salmonellosis as well as with the results from experimental studies (9,28,34). According to these authors Salmonella spp. induce IL-12 production from macrophages and dendritic cells after internalization into them. IL-12, on its turn, stimulates IFN-γ production from Th1 and NK cells, and TNF-α synthesis from monocytes, macrophages, NK and B cells (8,22,28). IFN-γ with the endotoxin, even in minimal concentrations, induces synthesis of IL-12 and regulates its effect upon NK and T cells (6,9,28). IL-12 and IFN-γ mutually amplify their effect which is inhibited by IL-4 and IL-10 (28,38). IL-12 enables proliferation and functional activity of NK cells and stimulates synthesis of pro-inflammatory cytokines (27). IFN-γ activates macrophages which undergo a number of biochemical and functional changes leading to their ability for cytokine produc tion (IL-1, IL-6, IL-8, TNF-α) and increased cytotoxicity (28).

According to our data, the correlation between TNF-α and IL-1β serum levels, and between TNF-α and IL-6 is also significant. This finding correlates with the results of other authors, who have established that IL-1 and IL-6 mediate the pathological effects of TNF-α (14). In patients with typhoid fever, a significant increase of TNF-α and IL-6 has been found and these concentrations correlated with the severity and fatal outcome of the disease (3).

A significant directly proportional correlation was established in our patients in terms of IL-1β and IL-ra serum concentrations. This might be explained with the fact that IL-1 induces the synthesis of its natural inhibitor (2,7,32). The attachment of IL-ra to their common receptor on the target cells switches off the action of IL-1 and protects the body from its harmful effects (10). It is accepted that receptor saturation with IL-ra is possible at ratio 10:1 between IL-ra and IL-1 (7).

The data concerning the significant and directly proportional correlation between IL-1β and IL-10 are intriguing. It is well known that IL-10 is the most potent anti-inflammatory cytokine inhibiting IL-12, IFN-γ, TNF-α and IL-1 synthesis, and reducing the capability of target cells for response to these cytokines (18,19,33). In terms of this fact, the converse correlation between IL-1β and IL-10 would have been more logical. Experimental Salmonella infection in mice has shown dynamics in IL-10 levels, similar with that of pro-inflammatory cytokines, and providing regulatory mechanism in vivo on their effect (1,17,18,19,33).

Correlation between serum IL-1β, IL-6 levels and electrolytes. The moderate and conversely proportional correlation between IL-1β and Na+, as well as between IL-6 and Na+ and Cl- in sera of our patients, can be explained with the inflammatory effect of these cytokines and the great loss of Na+ and Cl- in acute and pronounced inflammatory response. Some investigators believe that IL-1 has a crucial role in the origin of diarrheal syndrome in salmonellosis and direct secretory effect on intestinal epithelium (11,26). According to others, this cytokine stimulates target cells to produce prostaglandines and other pro-inflammatory mediators which activate the intestinal epithelial cells (30,32).
Cytokines are multifunctional molecules and the actions of some of them overlap to a certain extent. The network and the conditions in which they play, are characterised by many potentially synergistic and antagonistic effects between them as well as with many possibilities for internal control.

REFERENCES

Correlation of serum cytokines with the body temperature. Our data established significant and direct correlation between serum IL-1β, TNF-α and IL-6 levels with temperature. IL-1 and TNF are the main endogenous pyrogens. They act synergistically and amplify mutually causing fever, febrile reaction, muscle proteolysis, anorexia and hypoglycemia (30).

Cytokines are multifunctional molecules and the actions of some of them overlap to a certain extent. The network and the conditions in which they play, are characterised by many potentially synergistic and antagonistic effects between them as well as with many possibilities for internal control.

| Significant correlation between tested cytokines and basic clinical and laboratory parameters |  
| Pearson coefficient (rxy) | 0.9 – 0.7 | 0.7 – 0.5 | 0.5 – 0.3 |
| Parameters | direct | converse | direct | converse | direct | converse |
| IL-12/IFN-γ |  | IL-10/IL-6 |  |  |  |  |
| IL-12/TNF-α |  | TNF-α/IL-6 |  |  |  |  |
| IL-1β/IL-10 |  |  | IL-1β/IL-6 |  |  |  |
| IL-1β/IL-1ra |  |  |  | IL-10/IL-6 |  |  |
| IL-β/IL-6 |  |  |  |  | IL-β/IL-6 |  |  |
| IL-1α/IL-10 |  |  |  |  |  | IL-1α/IL-6 |  |

TABLE 1

<table>
<thead>
<tr>
<th>Type of proportion</th>
<th>0.9 – 0.7</th>
<th>0.7 – 0.5</th>
<th>0.5 – 0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α/IL-1ra</td>
<td>direct</td>
<td>converse</td>
<td>direct</td>
</tr>
<tr>
<td>IL-10/IL-6</td>
<td>direct</td>
<td>converse</td>
<td>direct</td>
</tr>
<tr>
<td>TNF-α/IL-6</td>
<td>direct</td>
<td>converse</td>
<td>direct</td>
</tr>
<tr>
<td>IL-1β/IL-6</td>
<td>direct</td>
<td>converse</td>
<td>direct</td>
</tr>
</tbody>
</table>

Correlation of serum cytokines with the body temperature. Our data established significant and direct correlation between serum IL-1β, TNF-α and IL-6 levels with temperature. IL-1 and TNF are the main endogenous pyrogens. They act synergistically and amplify mutually causing fever, febrile reaction, muscle proteolysis, anorexia and hypoglycemia (30).