Tumor Necrosis Factor-α and Interleukin-10 in Viral and Bacterial Gastroenteritis in Children

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Background: Gastroenteritis is a common cause of hospitalization and is associated with high morbidity in children. C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) are primary mediators of inflammation, and have been implicated in many infectious and non-infectious inflammatory diseases. The main objective of this study was to identify serum markers in viral and bacterial gastroenteritis.

Methods: Thirty-one patients admitted to a pediatric infection ward with gastroenteritis and definite pathogens were enrolled in the study: 17 patients had viral gastroenteritis and 14 bacterial gastroenteritis. Serum levels of TNF-α, IL-10 and CRP were measured in these 31 patients, and in a control group of 15 healthy children.

Results: Serum concentrations of TNF-α and CRP were significantly greater in patients with bacterial gastroenteritis than in patients with viral gastroenteritis and healthy controls ($p < 0.001$). Concentrations of IL-10 were increased, but not significantly, in patients with viral or bacterial gastroenteritis ($p = 0.577$ vs controls). Regarding diagnosis, the measurement of TNF-α and CRP levels was 78.6% and 92.0% sensitive, respectively; and 88.2% and 58.8% specific, respectively.

Conclusion: Serum TNF-α concentration may be a useful marker for distinguishing between viral and bacterial gastroenteritis. [*J Chin Med Assoc* 2005;68(6):250–253]

Key Words: C-reactive protein, gastroenteritis, interleukin-10, tumor necrosis factor-α

Introduction

Gastroenteritis is a common infection with high morbidity in children. Viruses, particularly rotaviruses, are the principal causes of childhood gastroenteritis,[1] but bacterial diarrhea is not uncommon.[2–4] Bacteria can be identified by culture 48 hours or more after stool sample collection. However, regarding the clinical features of gastroenteritis, it is difficult to distinguish clinically between viral and bacterial causes.[5] Patients with severe episodes of infection are at risk of systemic inflammatory response syndrome, which is known to induce the production of proinflammatory cytokines such as interleukins (ILs) and tumor necrosis factor-α (TNF-α).[6]

Proinflammatory cytokines, such as C-reactive protein (CRP), TNF-α, and IL-10 play a major role in coordinating mechanisms to control inflammation.[7] CRP, an acute phase reactant that increases in the presence of inflammation, infection or tissue injury, is synthesized in the liver.[8] TNF-α, a primary mediator of inflammation, has been implicated in several infectious and non-infectious inflammatory diseases,[9,10] and is a particularly useful marker of adequate host defense mechanisms, and for distinguishing viral from bacterial infections.[11–13] IL-10 inhibits the antigen- or mitogen-activated synthesis of several cytokines from different cells.[14] It also exerts immunostimulatory effects on B cells, cytotoxic T cell development, and thymocytes.[15] However, exact relationships between these cytokines and gastroenteritis remain unclear. The present study was designed to compare the

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concentrations of CRP, TNF-α, and IL-10 in patients with viral and bacterial gastroenteritis, and to try to identify accurate tools for differentiating between these 2 conditions.

Methods

Study population and protocol

Thirty-one children, of mean age 24.5 months (age range, 2–56 months), admitted to the pediatric infection ward of Taipei Veterans General Hospital with gastroenteritis and definite pathogens were enrolled in this study. The children were all diagnosed with gastroenteritis and were admitted after evaluation of clinical symptoms, including fever, vomiting, diarrhea, or the presence of blood in stools. Rotavirus was identified by the Premier™ Rotaclone® assay (Meridian Bioscience Inc, Cincinnati, OH, USA), an enzyme immunoassay intended for the detection of rotavirus antigen in human fecal specimens, while bacterial pathogens were identified by routine stool culture. Concentrations of CRP, TNF-α, and IL-10 were determined in all patients, whose sera were available on the first day of admission. CRP was measured in all patients, from blood samples taken on admission, by commercially available immunonephelometry (Behring Diagnostics Inc, San Jose, CA, USA). Other blood samples were centrifuged, and sera were stored at –70°C until assay.

None of the children were immunocompromised or had an underlying disease, and all lived with their families. No patients received corticosteroids or other immunosuppressive drugs during hospitalization. Eight boys and 7 girls, of mean age 21.8 months (age range, 6–46 months), comprised a group of healthy controls.

Cytokine assays

Serum levels of TNF-α and IL-10 were measured in duplicate by commercially available enzyme-linked immunosorbent assay (R&D Systems Inc, Minneapolis, MN, USA). All assays for TNF-α and IL-10 employed the quantitative sandwich enzyme immunoassay technique, with a specific monoclonal antibody specific for TNF-α pre-coated onto a microplate. We followed the methods and procedures for cytokine assays as described in the respective instruction booklets.

Statistical analysis

All results are expressed as mean ± standard deviation. Kruskal-Wallis tests were used to compare differences between patients and controls. An analysis of receiver operating characteristic (ROC) curves was performed to determine the sensitivity and specificity of measurement procedures for CRP, TNF-α and IL-10 regarding accurate differentiation of viral from bacterial gastroenteritis. SPSS version 10.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. For all tests, a p value of less than 0.05 was considered statistically significant.

Results

The following pathogens were identified in 31 children hospitalized with viral or bacterial gastroenteritis: rotavirus (n = 17); *Salmonella* spp. (10); *Aeromonas* spp. (2); *Shigella* spp. (1); and *Yersinia* spp. (1). Clinical features for the patients are shown in Table 1. Blood samples were collected on the first day of admission, and mean serum levels of CRP, TNF-α and IL-10 in the 3 study groups are shown in Table 2.

Among the total 46 study participants, age and gender distributions were not significantly different between the patient and control groups. However, the mean CRP level in patients with bacterial gastroenteritis (10.3 ± 6.3 mg/dL) was significantly greater (p < 0.001) than corresponding levels in patients with viral gastroenteritis (2.1 ± 2.3 mg/dL) and controls (1.6 ± 1.5 mg/dL). The mean TNF-α concentration in patients with bacterial gastroenteritis (29.9 ± 74.1 pg/mL) was also significantly greater

Table 1. Clinical features in 31 children with viral or bacterial gastroenteritis

	Viral gastroenteritis (n = 17)	Bacterial gastroenteritis (n = 14)
Age, mo	24.2 ± 15.5	24.7 ± 15.5
Males/females, n	9/8	7/7
Duration of symptoms, d	3.6 ± 1.6	3.9 ± 1.5
Vomiting, n (%)	13 (76.5)	3 (21.4)
Diarrhea, n (%)	17 (100)	14 (100)
Fever (> 38°C), n (%)	13 (76.5)	11 (78.6)
Seizure, n (%)	1 (5.9)	0 (0)

Table 2. Serum levels of C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) in children with gastroenteritis and in healthy controls

	Viral gastroenteritis (n = 17)	Bacterial gastroenteritis (n = 14)	Controls (n = 15)
CRP, mg/dL	2.1 ± 2.3*	10.3 ± 6.3*†	1.6 ± 1.5
TNF-α, pg/mL	5.0 ± 1.6	29.9 ± 74.1*†	5.4 ± 1.1
IL-10, pg/mL	73.1 ± 122.7*	52.3 ± 72.4‡	5.8 ± 2.6

$p < 0.001$ vs controls; †$p < 0.001$ vs viral gastroenteritis group; ‡$p = 0.004$ vs controls. Data are expressed as mean ± standard deviation.

Table 3. Predictive values of serum C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) when used as diagnostic tests to distinguish between viral and bacterial gastroenteritis

	CRP ≥ 2 mg/dL	TNF-α ≥ 7 pg/mL	IL-10 ≥ 10 pg/mL
Sensitivity, %	92.0	78.6	78.5
Specificity, %	58.8	88.2	29.4
Positive predictive value, %	68.4	88.6	47.8
Negative predictive value, %	84.6	83.3	62.5

($p < 0.001$) than corresponding concentrations in patients with viral gastroenteritis (5.0 ± 1.6 pg/mL) and controls (5.4 ± 1.1 pg/mL). Although mean IL-10 concentrations in patients with bacterial (52.3 ± 72.4 pg/mL) or viral gastroenteritis (73.1 ± 122.7 pg/mL) were significantly greater ($p \leq 0.004$) than the mean value in controls (5.8 ± 2.6 pg/mL), there was no statistically significant difference between the 2 patient groups ($p = 0.58$).

Serum CRP, TNF-α, and IL-10 concentrations were compared between the 2 gastroenteritis groups, and the control group, to assess the effectiveness of such markers as diagnostic tools. ROC curve analyses revealed area under the curve values of 0.912 for CRP, 0.935 for TNF-α, and 0.450 for IL-10. Thus, CRP and TNF-α are good diagnostic tools for distinguishing between viral and bacterial causes of gastroenteritis, whereas IL-10 is not.

The most appropriate cut-off diagnostic values for TNF-α and IL-10, as indicated by ROC curves, were used. For CRP alone, a cut-off diagnostic value of ≥ 2 mg/dL was used. Predictive values of serum CRP, TNF-α, and IL-10 levels when used as diagnostic markers to distinguish between viral and bacterial gastroenteritis (i.e. to indicate when a case of acute diarrhea is of bacterial rather than viral origin) are listed in Table 3.

Discussion

Diarrhea is one of the most common gastrointestinal illnesses, accounting for several billion cases annually worldwide, with 10–15 million deaths each year in the developing countries of Asia, Africa, and Latin America.[16] Early identification of the cause is important to prevent unnecessary antibiotic treatment, community outbreaks, and nosocomial transmission. Clinical criteria and rapid fecal tests, such as occult blood and fecal leukocytes, may give contradictory results and are not reliable as screening tests in infectious diarrhea.[17] As the results of microbiologic tests are available only after several days, detection of infections will continue to rely on the assessment of multiple markers. CRP has been useful in predicting the positivity of bacterial infection, and may therefore be helpful in targeting some patients with severe bacterial infection after hospital admission.[18,19] Indeed, the CRP results from previous studies[18,19] were compatible with our findings of a significant difference between patients with bacterial gastroenteritis and rotavirus gastroenteritis, and between patients with gastroenteritis and healthy controls.

Our data showed a significantly greater serum concentration of TNF-α in patients with bacterial gastroenteritis than in patients with rotavirus gastroenteritis and healthy controls. Similarly, a previous study[20] documented a higher plasma level of TNF-α in children with diarrhea than in uninfected controls. These results may suggest that TNF-α can distinguish clinically between bacterial and viral gastroenteritis in the acute phase, and that TNF-α may continue to increase during a prolonged episode of bacterial gastroenteritis. TNF-α expression increased in previous studies of salmonellosis,[21,22] and our data indicate that TNF-α plays an important role in host

defense against bacterial gastroenteritis. Further, TNF-α indicated the presence of bacterial infection with high sensitivity and specificity, and can thus be a useful parameter for distinguishing between bacterial and viral gastroenteritis in acute-phase illness.

The pleiotropic effects of IL-10 suggest that this cytokine has both proinflammatory and anti-inflammatory activity.[23] Few studies have evaluated IL-10 in patients with gastroenteritis, but expression of the cytokine is strongly increased in the intestinal tract, spleen, and liver during salmonellosis.[24] Westerholt et al[25] also found significantly increased IL-10 levels in patients with hemolytic uremic syndrome compared with controls. However, marked down-regulation of IL-10 production in patients with hemolytic uremic syndrome versus patients with gastroenteritis indicated an imbalanced immune response in hemolytic uremic syndrome.[25] These results, consistent with our own data, revealed high IL-10 levels in both viral and bacterial gastroenteritis compared with a control group, thus suggesting a systemic response in gastroenteritis. Nevertheless, as a diagnostic tool, measurement of IL-10 levels does not permit differentiation between bacterial and viral causes of gastroenteritis.

In summary, our results suggest that determination of serum CRP and TNF-α concentrations may allow earlier distinction than previously between bacterial and viral causes of gastroenteritis, whereas serum levels of IL-10 appear to be of no relevance in this regard. Further studies are needed to investigate the possibility of including serum measurements of other proinflammatory cytokines in the early differential diagnosis of bacterial versus viral gastroenteritis.

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