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Leucine-rich alpha-2 glycoprotein in the cerebrospinal fluid is a potential inflammatory biomarker for meningitis

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ABSTRACT

Background: Leucine-rich alpha-2 glycoprotein (LRG) is a novel biomarker for inflammatory diseases. We evaluated the levels of LRG, interleukin (IL)-6, and tumor necrosis factor (TNF)-α in the cerebrospinal fluid (CSF) of children with meningitis.

Methods: CSF samples from 10 patients with bacterial meningitis (BM) and 10 with aseptic meningitis (AM) were evaluated. Samples from 10 patients with febrile status (FS) were used as controls. LRG levels were measured using a two-site enzyme immunoassay. IL-6 and TNF-α levels were measured using a multiplex bead-based assay. CSF examination of patients with BM at the convalescent stage was also conducted.

Results: LRG and TNF-α levels in patients with BM, and IL-6 levels in patients with BM and AM showed significant increase compared with those in FS. Patients with BM at the convalescent stage showed significantly diminished LRG and IL-6 levels. LRG and IL-6 levels in CSF were indicated to be effective predictors for BM (LRG, AUC = 0.91; IL-6, AUC = 0.85). Only LRG levels showed a significant difference between patients with BM and AM (AUC = 0.78, $P = 0.034$).

Conclusions: LRG level could be a sensitive inflammatory biomarker for inflammatory diseases of the central nervous system, comparable with IL-6 level.

1. Introduction

Leucine-rich alpha-2 glycoprotein (LRG) is an approximately 50-kDa glycoprotein that contains repetitive sequences with a leucine-rich motif [1]. It is secreted by neutrophils undergoing differentiation [2] and by liver cells [3]. Although the exact function of LRG remains unknown, its expression is upregulated in patients with acute inflammatory conditions. Serum LRG level has been reported to increase in patients with ulcerative colitis [4], rheumatoid arthritis [5], appendicitis [6], and Kawasaki disease [7]. However, the measurement of LRG levels in the cerebrospinal fluid (CSF) has not been attempted for the diagnosis of neuroinflammatory diseases.

Bacterial meningitis (BM) is an infectious disease characterized by high mortality and morbidity rates if not promptly treated [8]. An inflammatory response plays a prominent role in the pathogenesis of cerebral injury during meningitis and other central nervous system infections [9]. Cytokines are molecules involved in the modulation of inflammatory and immune responses. Furthermore, CSF levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were found to be sensitive and specific inflammatory markers for BM [10−12].

We conducted a preliminary study to evaluate the usefulness of LRG levels in CSF as potential biomarkers for bacterial and aseptic meningitis (AM), which are important neuroinflammatory diseases. We used febrile status epilepticus (FS) as a control. We compared the effectiveness of LRG level as an inflammatory biomarker with those of IL-6 and TNF-α levels, and documented the efficacy of LRG in differentiating between the diagnoses of various conditions.

Abbreviations: LRG, Leucine-rich alpha-2 glycoprotein; CSF, Cerebrospinal fluid; BM, Bacterial meningitis; AM, Aseptic meningitis; FS, Febrile status epilepticus; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; ROC, Receiver operating characteristic; AUC, Area under the curve; IQR, Interquartile range

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Table 1
Baseline characteristics of the study population.

Characteristic	BM (*N* = 10)	AM (N = 10)	FS (N = 10)
Demographics, history and clinical examination			
Age (years), median (IQR)	0.8 (0.6–2.2)	3.0 (0.1–6.5)	1.5 (1.2–2.0)
Sex (female)	7/10 (70%)	4/10 (40%)	4/10 (40%)
Weight (kg), median (IQR)	8.7 (8.0–12.4)	11.7 (4.7–18.8)	10.9 (10.5–12.0)
Gestational week, median (IQR)	40 (39–40)	38 (37–38)	39 (39–40)
Birth weight (kg), median (IQR)	3.4 (3.1–3.5)	3.1 (2.7–3.2)	3.1 (2.8–3.4)
Temperature (°C), median (IQR)	39.0 (38.2–39.2)	38.0 (37.6–38.2)	39.0 (38.6–39.2)
Clinical diagnosis/pathogen etiology	*Streptococcus pneumoniae* 30% *Haemophilus influenzae* 50% *Streptococcus agalactiae* 20%	Mumps virus 20% Echovirus 9 10% Echovirus 30 10% Unknown 60%	Upper respiratory infection 50% Lower respiratory infection 30% Gastrointestinal infection 20%
Laboratory results			
White blood cells (K/μL), median (IQR)	12.8 (8.4–16.9)	9.4 (8.3–11.9)	13.9 (12.3–14.7)
Neutrophils (%), median (IQR)	72.5(68.8–80.0)	55.5 (33.8–75.5)	70.3 (58.2–83.4)
C-reactive protein (mg/dL), median (IQR)	18.5(7.5–27.1)	0.18 (0.04–0.51)	0.4 (0.2–1.1)
Cerebrospinal fluid cell count (/3 μL), median (IQR)	6916 (2480–14,500)	258 (136–1073)	4 (2–8)
Cerebeospinal fluid neutrophils (%), median (IQR)	91.5 (85.0–92.8)	32.0 (14.0–63.3)	20.0 (0.0–23.8)
Cerebrospinal fluid protein (mg/dL), median (IQR)	129.5 (87.5–157.3)	51.8 (27.6–89.0)	18.5(12.8–21.4)
Cerebrospinal fluid glucose (mg/dL), median (IQR)	12.5 (3.8–48.2)	46.0 (42.5–52.5)	93.5 (83.3–104.8)

BM: bacterial meningitis, AM: aseptic meningitis, FS: febrile status epilepticus, IQR: interquartile range.

2. Materials and methods

2.1. Case selection and sample collection

CSF samples collected from febrile patients with meningitis or FS who were admitted to Fukuoka Children's Hospital from 2007 to 2013 were used in this study. Clinical diagnoses were made by the attending pediatricians and later confirmed by the examination of available clinical and microbiological information for the purpose of this study. The diagnosis of BM and AM was established in accordance with the results of white blood cell counts, biochemical analysis, and presence of bacteria in the CSF culture. Clinical and microbiological parameters indicative of BM included CSF pleocytosis ($> 5/mm^3$) with neutrophil predominance, protein concentration of > 40 mg/dL, CSF/blood glucose ratio of < 0.4, and positive Gram staining and CSF culture results. AM was confirmed in children with CSF pleocytosis ($> 5/mm^3$) with lymphocyte predominance, normal or slightly increased protein concentration, normal CSF/blood glucose ratio, and absence of bacteria on CSF culture. FS was defined as a febrile seizure of > 15 min, with no elevation in the CSF cell count and confirmed negative CSF culture. Specimens of CSF were collected using lumbar puncture after obtaining written informed consent from the patients' parents. For patients with BM, a follow-up CSF sample was obtained to evaluate the efficacy of the antibiotic therapy during the convalescent stage (mean number of days, 14; range, 4–21). Ethical approval for this study was given by the Institutional Review Board and Ethics Committee of the Fukuoka Children's Hospital, Japan (No. 159).

2.2. Biomarker assays

CSF samples were immediately frozen after collection and stored at −30 °C until analysis. Samples were gently mixed to avoid gradient effects and were centrifuged to remove any debris and cells before analysis according to the manufacturers' protocols. Commercially available sandwich enzyme-linked immunosorbent assays (ELISA) for human LRG (IBL, Fujioka, Japan) was used for the quantitation of LRG. The levels of IL-6 and TNF-α were measured using a multiplex bead-based assay (BD CBA Flex set, BD Biosciences, NJ, USA). The assay ranges for LRG, IL-6, and TNF-α were 1.56–100 ng/mL, 10–2500 pg/mL, and 10–2500 pg/mL, respectively. These assays were performed in duplicate.

2.3. Statistical analysis

Statistical analysis was performed using the software SPSS version 18.0 (IBM, Armonk, NY, USA). For multiple comparisons between numerical variables or proportions, the Kruskal–Wallis rank sum test or the proportion test was conducted. If there were significant differences, the pairwise Wilcoxon rank sum test or the pairwise proportion test was performed, with adjustments using Bonferroni method. The Wilcoxon signed-rank test was performed for BM samples at the acute and convalescent phases. The prediction of poor prognosis with CSF biomarkers levels was conducted using the Mann–Whitney test. A two-tailed *P*-value of < 0.05 was considered statistically significant. The receiver operating characteristic (ROC) curve was assessed, and the area under the curve (AUC) was used to diagnose and differentiate between each diagnostic group. The discriminative performance was considered good when AUC was > 0.75 and excellent when it was > 0.90. An optimum threshold value (cutoff point) was selected as the situation maximizing the Youden index.

3. Results

3.1. Patient characteristics

We collected 30 CSF samples from 30 patients (15 boys and 15 girls). Of these, 10 patients each had a final clinical diagnosis of BM, AM, or FS. Table 1 shows the background characteristics. The results were expressed as the median and interquartile range (IQR), unless specified otherwise. The median age of patients with BM, AM, and FS was 0.9 (0.6–2.9), 3.0 (0.1–6.5), and 1.5 (1.2–2.0) years, respectively. In patients with BM, the confirmed etiological agents were *Streptococcus pneumoniae* in three patients, *Haemophilus influenzae* type b in five, and group B *Streptococcus agalactiae* in two. In patients with AM, the pathological agents identified included Mumps virus in two patients and echoviruses 9 and 30 in one each. The viral etiology was unknown in the remaining six patients. Follow-up CSF samples were acquired during the convalescent phase for patients with BM.

3.2. CSF biomarkers in each diagnostic group

The CSF levels of LRG, IL-6, and TNF-α were compared between the diagnostic groups (Fig. 1). We found a statistically significant increase of LRG levels in CSF in the BM group (median, 374.5 ng/mL; IQR, 191.5–2725.8; $P = 0.014$) compared with the FS group (median,