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Cerebrospinal fluid eotaxin and eotaxin-2 levels in human eosinophilic meningitis associated with angiostrongyliasis

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Abstract

Eosinophilic meningitis associated angiostrongyliasis (EOMA) is a harmful disease of the brain and spinal cord caused by a parasitic helminth, *Angiostrongylus cantonensis*, presenting with severe headaches and cerebrospinal fluid (CSF) eosinophilia. However, the immunologic pathophysiology especially in relation to the eosinophilic inflammation is still unknown. We measured the CSF concentrations of eotaxin and eotaxin-2 of 30 patients and 10 controls. The CSF eotaxin and eotaxin-2 levels of the EOMA patients were significantly higher than those of the controls ($p < 0.001$). The positive detection values were 83.3% (25/30) and 93.3% (28/30) for eotaxin and eotaxin-2, respectively. CSF eotaxin-2 levels also correlated with CSF eosinophilia ($p = 0.002$). These results might indicate that the recruitment of eosinophils to the brain and spinal cord in EOMA patients could be related to elevated eotaxin-2 levels.

Keywords: Cerebrospinal fluid; Eotaxin; Eotaxin-2; Angiostrongyliasis; Eosinophilic meningitis

1. Introduction

Eosinophilic meningitis associated angiostrongyliasis (EOMA) is a harmful disease of the brain and spinal cord caused by a parasitic helminth, *Angiostrongylus cantonensis*. EOMA cases have been reported from different countries in Southeast Asia and the South Pacific islands [1–6]. Humans are non-permissive hosts who become infected by consuming infected larvae in snails, slugs, paratenic hosts or contaminated uncooked vegetables [6]. Infected patients present with severe headaches, vomiting, paresthesia, weakness and sometimes visual disturbances and extraocular muscle paralysis. Severe infection can lead to chronic, harmful disease and even death [3].

It is known that in *A. cantonensis*-infected patients the percentage of eosinophils among all leukocytes in the cerebrospinal fluid (CSF) is significantly higher than in the peripheral blood [2]. Which component of the brain of infected patients recruits eosinophils from the peripheral blood to the brain and spinal cord, as well as the immunological pathophysiology especially of the eosinophilic inflammation in EOMA are still unknown. To better understand the immunological pathogenesis of EOMA in infected humans, we determined the CSF concentration of eotaxin and eotaxin-2, two CC chemokines that are potent eosinophil chemoattractors and signal via the CC chemokine receptor 3 (CCR3). Thus they are chemokines with a high possibility of being related to the eosinophilic inflammation in patients with EOMA. The correlations between the concentrations of each eotaxin and CSF eosinophilia in infected patients were investigated.

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2. Patients and methods

Informed consent was obtained from all human adult participants and from parents or legal guardians of minors. The study protocol was approved by the Human Ethical Committee of Khon Kaen University (HE500230).

After sample collection on admission, all EOMA patients received standard corticosteroid treatment as previously described [7].

2.1. Eosinophilic meningitis associated angiostrongyliasis (EOMA)

CSF samples were obtained from 30 EOMA patients (24 males and 6 females, aged from 11 to 55 years; mean ± SD = 33.27 ± 11.09 years) at Loei Hospital, Nampong Hospital, and Srinagarind Hospital, Northeastern Thailand, from May 2004 to March 2006. The diagnostic criterion for EOMA was a finding of ⩾10% eosinophils in the CSF [7]. All patients presented with severe headaches after eating either raw snails or shrimps or monitor lizards (*Varanus bengalensis*). The mean incubation period ± SD was 18.89 ± 19.82 days (median = 14 days and range 1–90 days). The clinical symptoms included headache (30/30; 100%), neck stiffness (8/30; 26.7%), fever (16/30; 53.3%), nausea (19/30; 63.3%), vomiting (13/30; 43.3%), paresthesis (1/30; 3.3%) and extraocular muscle weakness (1/30; 3.3%). 21 of 30 (70%) patients showed peripheral blood eosinophilia and all patients presented with CSF eosinophilia (range 10%–84%). The mean CSF leukocyte level ± SD was 817 ± 719 wbc/μl (range 48–3244 wbc/μl). 17 of 30 (56.67%) patients had elevated CSF protein (>100 mg/dl). The CSF glucose was >40 mg/dl in 22 of 30 (73.33%) patients. All stains and bacterial cultures of the CSF turned out negative. All serum VDRL tests were non-reactive. All EOMA patients were positive for specific IgG antibody to *A. cantonensis* in the CSF by enzyme-linked immunosorbent assay (ELISA) [8]. The ELISA optical values in the CSF for the specific IgG antibody ranged from 0.247 to 2.750 (mean ± SD = 0.486 ± 0.735). Immunoblotting, as documented previously [9], revealed a specific *A. cantonensis* band at a molecular mass of 29 kDa in all 30 EOMA sera. The CSF samples were obtained immediately after admission to the hospital during the acute phase and were stored at −70 °C until use.

2.2. Control subjects

The control subjects ($n = 10$) consisted of four non-meningitis patients whose CSF was sampled during lumbar puncture for anesthesia before surgery and of six symptomatic meningitis like patients (tension headache) with normal CSF profiles. All control samples were negative for specific IgG antibody to *A. cantonensis* antigen in the CSF by enzyme-linked immunosorbent assay (ELISA) [8] and were non-reactive for the specific *A. cantonensis* antigenic band at a molecular mass of 29 kDa by immunoblotting [9].

2.3. Determination of eotaxin concentrations

The concentrations of CSF eotaxin and eotaxin-2 were measured by a quantitative sandwich enzyme immunoassay with intra- and inter-assay precision (R&D systems, Minneapolis, MN) according to the manufacturer's manual. Each CSF sample was determined in duplicate and the mean was used as reading value for the sample. Data analysis was carried out by generating a linear standard curve according to the manufacturer's instructions. The detection ranges of the kits were <5–1000 pg/ml (eotaxin) and 1.83–2000 pg/ml (eotaxin-2). Briefly, all eotaxins (eotaxin or eotaxin-2) in the samples or standards, bound to antibodies specific for individual homologous eotaxins that were coated in the wells of a microplate. After unbound components were removed by washing the well, horseradish peroxidase conjugated to a polyclonal antibody against the individual homologous eotaxins was added to the wells. After incubation for 2 h at room temperature, following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (tetramethylbenzidine) was added to the wells for the color development. A stop solution was then added to each well and the optical density was read at 450 nm, with the correction wavelength set at 540 nm.

2.4. Statistical analysis

Differences in the results were analyzed by the Mann–Whitney Rank Sum test with a p value less than 0.05 being decided on as significant. Correlations were analyzed using the Pearson Correlation test.

3. Results

The CSF eotaxin and eotaxin-2 concentrations of the control and EOMA patients are shown in Fig. 1 and Table 1. The CSF eotaxin and eotaxin-2 levels of the EOMA patients were significantly higher than those of the controls ($p < 0.001$) (Table 1). Fig. 2 shows a scatter diagram illus-

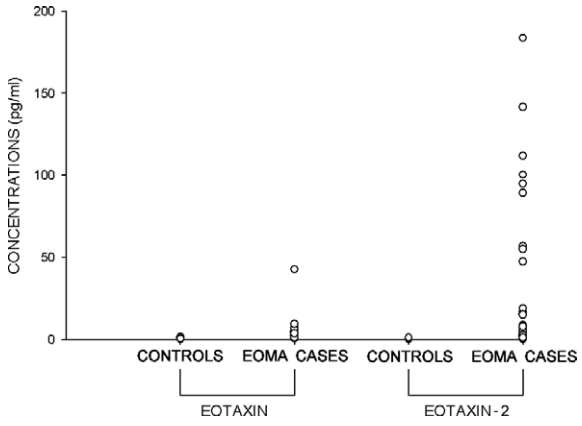

Fig. 1. Distribution of CSF CC chemokine levels of EOMA cases ($n = 30$) and control groups ($n = 10$).