

Differential fatty acid analysis of cerebrospinal fluid in infants and young children with suspected meningitis

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Abstract

Purpose Meningitis is relatively common in infants and young children and can cause permanent brain damage. The aim of this study was to determine whether meningitis is associated with fatty acids in cerebrospinal fluid (CSF).

Methods CSF samples from children between 3 months and 6 years of age admitted to the Tabriz public hospitals who met clinical criteria of meningitis were collected at enrollment. A total of 81 samples were analyzed for fatty acid profile by gas–liquid chromatography.

Results Children with a purulent meningitis demonstrated a higher percentage of oleic acid ($p < 0.05$, $>10\%$) and lower percentages of omega-3 polyunsaturated fatty acids ($p < 0.001$, $<-40\%$) than aseptic meningitis and nonmeningitis groups did. There was an inverse relationship between CSF long-chain omega-3 fatty acids and the total number of leukocytes and differential counts of neutrophils and lymphocytes in the purulent meningitis group. Moreover, significantly lower omega-3 fatty acids ($p = 0.001$, -37%) and higher ratio of n-6/n-3 ($p = 0.02$, -29%) were found in patients with purulent meningitis with sepsis than in those with meningitis and no sepsis.

Conclusions This study provides evidence that purulent meningitis and its complication with sepsis are associated with important disturbances in CSF fatty acids, mainly deficiency in long-chain omega-3 polyunsaturated fatty acids.

Keywords CSF · Docosahexaenoic acid · Eicosapentaenoic acid · Meningitis · Omega-3 fatty acids

Introduction

Deregulation of fatty acids has been implicated in neuroinflammatory diseases [1]. Content of fatty acids in cerebrospinal fluid (CSF) has been linked to various neurological/neurodegenerative disorders including blood–brain barrier dysfunction [2], Parkinson’s disease [3], and Alzheimer’s disease [4]. Moreover, clinical data support an association of central fatty acids with energy metabolism and metabolic homeostasis [5, 6].

Attempts to analyze meningitis CSF fatty acids have rather focused on identifying components of bacterial cells as possible biomarkers for rapid diagnosis of meningitis [7–9]. These studies assayed only a limited number of participants and fatty acids. To date, the comprehensive analysis of fatty acids in meningitis CSF has not been reported. However, infection diseases in general are characterized by disturbed fatty acid metabolism in the host that might contribute to the pathogenesis [10–12]. Therefore, new data on fatty acids in meningitis CSF are not only essential to better understand the pathophysiology but are also crucial for the development of effective interventions to prevent infection-induced metabolic disturbances and to improve treatment outcome.

The aim of this study was to investigate the differential fatty acid profile in CSF obtained from a group of infants and young children with suspected meningitis. We used gas–

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liquid chromatography–flame ionization detector to separate, identify, and quantify CSF fatty acids. Our findings indicated that the relative amounts of oleic acid were increased in purulent meningitis, whereas those of omega-3 polyunsaturated fatty acids (PUFAs) were decreased. Moreover, significant correlations were found between CSF immune cell profile and omega-3 fatty acids in purulent meningitis.

Material and methods

Subjects and specimens

All infants and young children were recruited from a patient population scheduled for lumbar puncture for symptomatic meningitis as part of their emergency evaluation at the Tabriz university hospitals (five public sector hospitals in Tabriz) between May 1, 2014, and June 30, 2016. In the present study, 81 patients (46 boys and 35 girls) were enrolled. This cross-sectional study was reviewed and approved by the ethics committee of Tabriz University of Medical Sciences (IRB permission number 5.4.11707), and a direct parent (mother or father) provided written informed consent. This study was performed according with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Patients younger than 3 months of age or over 6 years of age and with evidence for another infection, traumatic lumbar puncture, CNS disorders, oncologic disease, hepatitis, or diabetes that could influence CNS were excluded to obtain a more homogeneous study population. Patients were also excluded if they had received treatment with antibiotics, corticoids, or nonsteroidal anti-inflammatory drugs in the last 1 week of puncture. The initial antibiotic therapy was administered immediately after the lumbar puncture. All assays described below were performed blind of diagnosis.

Cerebrospinal fluid analysis

Cerebrospinal fluid samples were obtained from patients by lumbar puncture performed according to a standardized procedure. CSF samples were collected directly into sterile tubes for bacterial culture and diagnostic evaluation immediately after collection or frozen after centrifugation at $-70\text{ }^{\circ}\text{C}$ for fatty acid analysis. The clinical and laboratory diagnoses were made using a routine clinical assessment and CSF examination according to WHO guidelines [13]. CSF analysis included microscopy, differential cell count, glucose content, and measurement of protein. Patients or CSF samples with abnormal coloring or with more than 50 red blood cells (RBCs)/ mm^3 [14] were excluded from this study. All quantitative determinations of glucose and protein were performed by

standard commercial kits on a BT 3000 Autoanalyzer (Biotechnica Instruments, Italy).

Purulent meningitis was defined as clinical evidence of meningitis with laboratory confirmation of decreased CSF glucose (CSF/serum glucose $<50\%$) with pleocytosis of ≥ 100 leukocytes per cubic millimeter with $>80\%$ polymorphonuclear neutrophilic leukocyte ($n = 12$) or presence of infection in CSF culture or bacteria in direct examination of CSF ($n = 11$). Septic meningitis in nine patients was defined according to the ACCP/SCCM definitions [15]. Aseptic meningitis was defined as the acute onset of meningitis with mononuclear pleocytosis and the absence of any purulent meningitis criteria and included 23 patients. Children without CSF pleocytosis and any laboratory meningitis criteria were classified as control subjects; they included 35 patients of whom 9 had influenza and vaccination, while the remaining 26 had fever of unknown origin.

Chemicals and reagents

All solvents (hexane, methanol, chloroform, acetyl chloride, and potassium carbonate) were obtained from Merck Company (Darmstadt, Germany). Deionized water with resistivity of at least $18\text{ M}\Omega/\text{cm}$ was obtained from a Milli-Q water purification system (Millipore, Bedford, Mass, USA). The stock standards of tridecanoic acid and fatty acid methyl esters were purchased from Sigma Chemicals Company (St. Louis, USA). Working standard solutions of 0.1 to 300 $\mu\text{g}/\text{ml}$ were prepared freshly from the stock solution by sequential dilution with hexane and were kept under a nitrogen atmosphere in the dark at $4\text{ }^{\circ}\text{C}$.

Gas–liquid chromatography of CSF fatty acids

A hydrogen flame ionization detector (FID), equipped with a hydrogen generator (H2 NM Plus, LNI Schmidlin, Switzerland) and a Buck Scientific model 610 gas chromatograph (SRI Instruments, Torrance, USA) consisting of standard oven for temperature ramping, a split flow injector with a deactivated glass liner, a computer interface, and PeakSimple software version 3.59, were used for data collection and processing.

Total lipids were extracted from 1 ml of CSF samples by using the Bligh–Dyer method and were esterified with methanol during catalysis with acetyl chloride [16]. Tridecanoic acid (13:0, 100 $\mu\text{g}/\text{ml}$) was added to all the CSF samples as the internal standard element. Fatty acid methyl ester derivatives formed by isolated CSF lipids were separated on a highly polar biscyanopropyl polysiloxane capillary column (TR-CN100 $60 \times 0.25\text{ mm} \times 0.2\text{ }\mu\text{m}$ film thickness) from Teknokroma (Spain). Helium was used as the carrier gas, and column linear velocity was set at 20.0 cm/s. A high

injector temperature of 250 °C was used to convert all studied compounds into gaseous state before entering the column. The oven temperature started at 170 °C for 5 min and then ramped with 2 °C/min to 210 °C and then isothermal for 15 min. Peak retention times were identified by injecting known standards. The laboratory variability of the fatty acid analysis was established by repeated measures on a pooled CSF. The coefficient of variation was 3.2 % for palmitic acid (16:0), 4.1 for linoleic acid (18:2n-6), 9.4 for eicosapentaenoic acid (EPA), and 10.6 for docosahexaenoic acid (DHA).

Statistical analysis

The level of significance between group means was calculated according to *t* test or analysis of variance for continuous variables and χ^2 test for categorical variables. A *p* value <0.05 was considered statistically significant. The values of the leukocyte count were converted by logarithmic transformation to normalize distribution of the data. Spearman correlation coefficients were calculated between fatty acids and CSF parameters. Multivariate analyses were used to test the independence of associations between the presence of purulent meningitis as outcome variable and protein and fatty acid composition of CSF as independent variables. All analyses were carried out using SPSS for windows version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Eligible study participants were predominantly boys (60 %) with an average age of 1.34 years. Table 1 shows the demographic characteristics and CSF parameters of the three studied groups. There were no significant differences regarding

age and sex between the studied groups. Palmitic acid was the major fatty acid in CSF, followed by stearic acid and oleic acid in both groups (Table 2). The mean values of oleic acid was higher in patients with purulent meningitis than in patients with nonmeningitis as well as aseptic meningitis (>10 %, *p* < 0.05), reflecting higher total monounsaturated fatty acids in patients with meningitis. Patients with purulent meningitis showed significantly lower levels of α -linolenic acid, EPA, and DHA than did the nonmeningitis group and patients with aseptic meningitis (*p* < 0.01, Table 2). A significant difference was also seen for higher n-6 to n-3 polyunsaturated fatty acid (n-6/n-3) ratio in patients with purulent meningitis (*p* < 0.01, >1.98-fold). Overall, there were no significant differences in the other fatty acid and total polyunsaturated fatty acid levels between the studied groups.

There was no significant relation between age or sex and CSF fatty acids in the entire study sample or within the subgroups. Correlation coefficients between CSF laboratory parameters and the fatty acid content of CSF are shown in Table 3. In patients with purulent meningitis, CSF white blood cell (WBC) count and ratio of neutrophils to lymphocytes were inversely correlated with the long-chain omega-3 PUFA content and directly with the n-6/n-3 ratio of CSF. In a multivariate regression analysis that included CSF to serum glucose ratio, the inverse association of purulent meningitis WBC count with omega-3 PUFA ($\beta = -0.12$, CI = 0.04–0.33) and the direct association with the n-6 to n-3 ratio ($\beta = 4.89$, CI = 2.27–10.57) remained significant (*p* < 0.05), but as expected, these associations did not remain significant when controlling for other CSF parameters including protein concentration and ratio of neutrophils to lymphocytes.

Subjects were divided into two groups according to the presence of septicemia that was seen in 9 of 23 subjects with purulent meningitis to further investigate the association of

Table 1 Clinical characteristics and laboratory parameters of cerebrospinal fluid of patients with suspected meningitis

	Nonmeningitis (<i>n</i> = 35)	Aseptic meningitis (<i>n</i> = 23)	Purulent meningitis (<i>n</i> = 23)
Age, years	1.00 (1.04–1.63)	0.85 (0.91–1.80)	1.08 (1.03–1.63)
Sex, boys/girls	20/15	13/10	13/10
WBC count, /mm ³	1 (0.98–1.80)a	1375 (1718–2496)b	2500 (3180–5105)c
N/L	0.95 ± 0.13a	0.40 ± 0.15a	8.33 ± 4.79b
Protein, mg/dl	32.71 ± 9.70a	37.61 ± 8.84a	154.39 ± 75.61b
Glucose, mg/dl	64.00 ± 13.79a	58.57 ± 14.09a	46.61 ± 20.86b
CSF/serum glucose	0.70 ± 0.13a	0.68 ± 0.14a	0.55 ± 0.24b

Continuous variables are presented as mean ± SD or, if variables did not exhibit normal distribution, medians with 25 and 75 percentiles in brackets and compared by the Tukey test for pairwise comparisons between the three groups, whereas categorical variables are presented as frequencies and compared by the Chi-square test. The patients were classified according to established clinical guidelines based on cerebrospinal fluid analysis and culture (see “Methods” section). Values in the same row with different lowercase letters are significantly different, *p* < 0.05

N/L ratio of neutrophils to lymphocytes, WBC white blood cell

Table 2 Cerebrospinal fluid fatty acid composition in patients with suspected meningitis

	% Of total fatty acids		
	Nonmeningitis (<i>n</i> = 35)	Aseptic meningitis (<i>n</i> = 23)	Purulent meningitis (<i>n</i> = 23)
16:0 (palmitic acid)	42.64 ± 5.44	43.12 ± 3.70	40.62 ± 3.35
16:1n-7 (palmitoleic acid)	2.15 ± 0.29	1.96 ± 0.42	2.16 ± 0.62
18:0 (stearic acid)	25.18 ± 5.22	25.25 ± 3.40	26.35 ± 2.60
18:1n-9 (oleic acid)	16.62 ± 2.55a	16.12 ± 2.58a	18.40 ± 2.75b
18:2n-6 (linoleic acid)	5.79 ± 1.20	5.57 ± 1.84	6.03 ± 1.82
18:3n-3 (α-linolenic acid)	1.76 ± 0.54a	1.82 ± 0.32a	1.30 ± 0.33b
20:4n-6 (arachidonic acid)	3.46 ± 0.74	3.67 ± 1.22	3.93 ± 1.04
20:5n-3 (eicosapentaenoic acid)	1.65 ± 0.31a	1.72 ± 0.62a	0.83 ± 0.48b
22:6n-3 (docosahexaenoic acid)	0.75 ± 0.15a	0.77 ± 0.22a	0.38 ± 0.29b
Saturated FAs	67.82 ± 2.75	68.37 ± 2.97	66.97 ± 3.31
Monounsaturated FAs	18.77 ± 2.59a	18.08 ± 2.78a	20.56 ± 2.79b
S/P ratio	5.17 ± 0.84	5.20 ± 0.97	5.56 ± 1.24
n-3 polyunsaturated FAs	4.16 ± 0.80a	4.30 ± 0.89a	2.51 ± 0.95b
n-6/n-3 ratio	2.27 ± 0.42a	2.25 ± 0.73a	4.49 ± 1.63b

Values are mean ± SD. Detection limit was 0.05 % of the total area. The patients were classified according to established clinical guidelines based on cerebrospinal fluid analysis and culture (see “Methods”). Means in the same row with different lowercase letters are significantly different, $p < 0.05$ (Tukey test for pairwise comparisons between the three groups)

FAs fatty acids, S/P saturated to polyunsaturated fatty acids

fatty acids with purulent meningitis. There were significant differences in omega-3 PUFA between the groups. Patients with purulent meningitis and sepsis showed lower amounts

of omega-3 PUFA (−38 %, $p = 0.001$) and higher levels of n-6 to n-3 PUFA (41 %, $p = 0.02$) than did patients with purulent meningitis without sepsis (Fig. Fig. 1).

Table 3 Correlations between cerebrospinal fluid fatty acids and cerebrospinal fluid parameters

	ln WBC count	N/L	Protein	CSF to blood glucose
Nonmeningitis				
18:1n-9 (oleic acid)	0.003	0.342	0.244	−0.043
18:3n-3 (linolenic acid)	0.013	0.114	0.168	0.091
20:5n-3 (eicosapentaenoic acid)	−0.025	−0.285	0.102	0.241
22:6n-3 (docosahexaenoic acid)	0.092	0.143	−0.123	0.218
n-6/n-3 ratio	−0.010	−0.114	−0.082	−0.268
Aseptic meningitis				
18:1n-9 (oleic acid)	0.108	0.242	0.211	−0.164
18:3n-3 (α-linolenic acid)	−0.103	−0.266	−0.198	0.193
20:5n-3 (eicosapentaenoic acid)	−0.328	−0.323	−0.098	0.210
22:6n-3 (docosahexaenoic acid)	−0.303	−0.242	−0.246	0.046
n-6/n-3 ratio	0.300	0.348	0.134	−0.294
Purulent meningitis				
18:1n-9 (oleic acid)	0.203	−0.178	0.107	0.027
18:3n-3 (linolenic acid)	−0.340	−0.077	−0.335	0.413
20:5n-3 (eicosapentaenoic acid)	−0.454*	−0.516*	−0.230	−0.360
22:6n-3 (docosahexaenoic acid)	−0.786**	−0.341	−0.398	−0.021
n-6/n-3 ratio	0.443*	0.394	0.339	0.244

The Spearman correlation coefficients (rho values) are displayed

ln natural logarithm, N/L ratio of neutrophils to lymphocytes, WBC white blood cell

* $p < 0.05$; ** $p < 0.01$

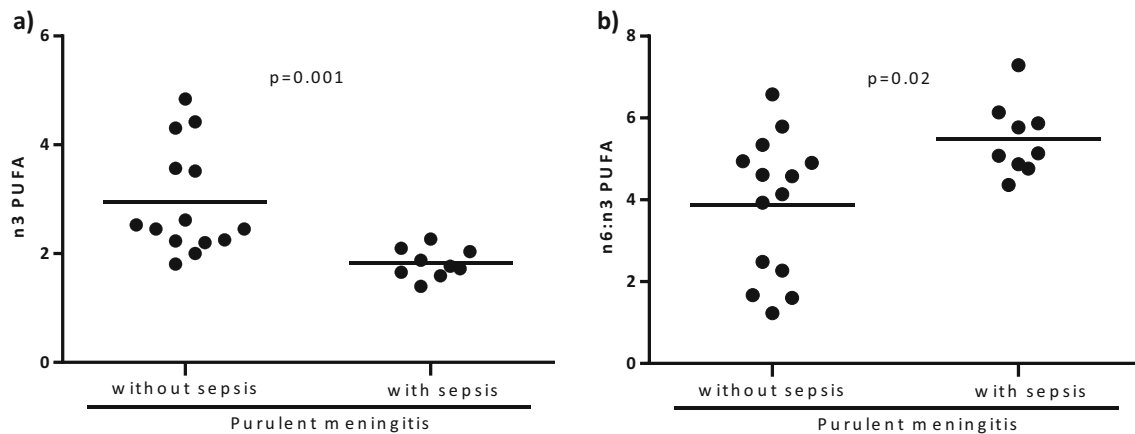


Fig. 1. Dot-graph showing the mean of n-3 polyunsaturated fatty acids (PUFAs) (a) and n-6 to n-3 PUFA ratio (b) in the cerebrospinal fluid between purulent meningitis with ($n = 9$) and without (14) sepsis.

Discussion

Lipid abnormalities are typical features of many infectious diseases [17, 18]. These changes in lipids are of fundamental importance in the pathology of infection and subsequent induction of inflammatory response. Natural fatty acids are the main constituents of lipids and are biomarker candidates of spinal fluid [19, 20]. Preliminary studies have shown that the fatty acid composition of spinal fluid is useful for the diagnosis of meningitis in animals and humans [7–9]. To our knowledge, this is the first report on the comprehensive profile of fatty acids in CSF samples that evaluated dysregulated lipid metabolism associated with meningitis in infants and young children.

Consistent with previous reports [7], palmitic and stearic acids were the principal fatty acids in human CSF. Few studies have reported the fatty acid profile of human spinal fluid [1, 21–23], presumably because of limitation of sampling. While a limited fatty acid types were analyzed, these studies have clearly shown that the CSF fatty acid profile is significantly modified in response to conditions associated with neuropathology.

Oleic acid was markedly elevated in the purulent meningitis group as compared to patients without meningitis or aseptic meningitis. Some previous reports suggest that high monounsaturated fatty acids, oleic acid in particular, might be involved in the pathogenesis of bacterial infectious diseases [10–12]. Animal models demonstrated a significant increase in the tissue content of oleic acid in response to an experimental infection [24]. In agreement with our data, a significant increase in plasma oleic acid, accompanied by a decrease in PUFA, has been reported in septic patients [25].

Data accumulated from various models have proven that omega- or n-3 fatty acids such as α -linolenic acid and DHA are crucial for the brain growth and protection against neuronal injuries [26]. In this study, CSF levels of omega-3

PUFA were reduced in patients with purulent meningitis and even more so in patients with purulent meningitis complicated with sepsis. Since omega-3 fatty acids are essential in human nutrition, they are locally available in CSF primarily through transport from the blood–brain barrier (BBB). Our results are suggestive of disrupted omega-3 fatty acid passage through the BBB in purulent meningitis. PUFAs are specifically transported from the plasma pool into the brain via a selective mediated mechanism, which has not yet been fully elucidated [27]. Studies using animal models of bacterial infection derived from pigs with meningitis have demonstrated that acute infection markedly decreases omega-3 PUFA and increases omega-6 PUFA in major internal organs [28]. Accordingly, the current study showed that the levels of omega-3 PUFA were negatively correlated with spinal fluid pleocytosis in patients with purulent meningitis. However, the relationship of omega-3 PUFA with CSF parameters was not significant in the aseptic meningitis and control groups, which may reflect a major role of omega-3 PUFA in high-grade inflammatory state associated with bacterial infection.

Perhaps the key implication here is that interventions are already underway to improve neurological function through omega-3 PUFA administration in infection or other inflammatory conditions [29, 30]. Thus, omega-3 PUFA deficiency secondary to bacterial infection may represent an important therapeutic target for management of infants and children with purulent meningitis.

To conclude, the composition of fatty acids within the CSF of infants and young children with purulent meningitis was modified when compared to controls. Specifically, these patients showed a significantly higher oleic acid and lower omega-3 PUFA than the aseptic meningitis and control groups did. This disturbance in the central fatty acids may be detrimental, particularly leading to excessive inflammation in the CNS due to bacterial infection.

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Authors' contribution Conceived and designed study: MD and MB. Performed measurements: EE, AM, MS, and SG. Analyzed the data: EE, MB, and AM. Contributed reagents/materials: EE, AM, MS, SG, and AA. Wrote the paper: MD, MB, EE, AM, MS, AA and SG. Critically revised the manuscript: MD and MB.

Compliance with ethical standards

This cross-sectional study was reviewed and approved by the ethics committee of Tabriz University of Medical Sciences (IRB permission number 5.4.11707), and a direct parent (mother or father) provided written informed consent. This study was performed according with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Conflict of interest The authors declare that there is no conflict of interest.

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