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Relative expression of fibroblast growth factor-1 in the cerebrospinal fluid of patients with bacterial meningitis; A Western Blot analysis

Zivar Salehi* and Lida Gholizadeh

Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

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ABSTRACT

Meningitis is an inflammation of the membranes that surround the brain and spinal cord, thereby involving the arachnoid, the pia and the cerebrospinal fluid (CSF). It is divided into viral and bacterial meningitis. For different reasons the diagnosis of bacterial meningitis is very important. The examination of CSF samples may provide information about causative microorganism. The sensivity of Gram-stained specimen of CSF ranges from 60% to 90%. CSF is continuously secreted by the choroids plexus and contains growth factors which are present under specific pathological conditions. As CSF is in close contact with the extracellular space of the brain, biochemical brain modifications could be reflected in the CSF and study of growth factor expression in the CSF might identify biomarkers of meningitis. As fibroblast growth factor-1 (FGF-1) is important in neural cell survival, we studied the changes in the total protein concentration (TPC) and FGF-1 expression in the CSF of normal control and patients with meningitis using Western blott. No significant increase in the CSF TPC in the patients with bacterial meningitis has been seen when compared to control group. However, significant increase in the CSF FGF-1 expression in the patients with meningitis has been seen as compared to control group. It is suggested that FGF-1 could be significantly involved in the pathophysiology of meningitis. we have also conclude that the FGF level in the CSF may provide additional information in the differential diagnosis of meningitis.

1. Introduction

The central nervous system (CNS) infections are classified as meningitis and encephalitis (Scheld et al., 1989). Meningitis is one of the most common infectious CNS syndromes, defined as an inflammation of the meninges. Viruses or bacteria can cause acute meningitis of infectious etiology. Meningitis is clinically categorized into an acute and chronic disease based on the acuity of symptoms. Acute meningitis develops over hours to days, while in chronic meningitis symptoms evolve over days or even weeks (Leib and Tuber, 1999). The clinical

*Corresponding author. Dr. Zivar Salehi
Tel: +989113337003; Fax: +981313233647
E-mail address: geneticzs@yahoo.co.uk

symptoms are fever, malaise, and vomiting. These signs are poorly sensed in adults (Thomas et al., 2002). Signs of meningeal irritation are rare among younger children. Small children can present other signs, such as an inability to feed, drowsiness, vomiting, and bulging fontanel (de Almeida et al., 2007). Acute meningitis with infectious etiology is viral or bacterial. From the first months of life, the *H. influenzae*, *N. meningitides* and *S. pneumonia* are responsible for 70 to 90% of the cases of acute bacterial meningitis in all regions of the world (Schlech, 1992). Infections by *H. influenzae* have been significantly reduced because of systematic vaccination. Chronic meningitis of infectious cases is caused by tuberculosis, fungus, syphilis and histoplasmosis (Wilhelm and Ellner, 1986).

Bacterial meningitis is a severe, potentially life-threatening infection that is associated with high rates of morbidity and significant disability in survivors. In recent years, despite improvements in antimicrobial therapy and intensive care support, overall mortality rates related to bacterial meningitis of around 20% have been reported (Sigurdardottir et al., 1997). Viral infections of the CNS are common occurrences in clinical practice; however the incidence of these cases is not well defined (Big et al., 2009).

The examination of the cerebrospinal fluid (CSF) is a cornerstone in diagnostic procedure for patients with suspected meningitis. CSF is secreted continuously by the choroids plexus, located in the lateral, third and fourth ventricles. A number of studies have identified CSF as a carrier of important cytokines, such as brain derived neurotrophic factor (BDNF), insulin like growth factor (IGF), transforming growth factor (TGF), fibroblast growth factor (FGF) and other growth factors which are present under specific physiological and pathological conditions (Mashayekhi and Salehi, 2005). In recent years brain specific proteins as markers for structural brain damage have been widely investigated. There seems to be a close relation between the concentrations of these growth factors and cytokines in the CSF and the severity of brain damage (Mashayekhi and Salehi, 2005; Mashayekhi and Salehi, 2006; Mashayekhi et al., 2010). Elevated levels of stem cell factor (SCF) have been reported in the CSF of children with meningitis (Mashayekhi and Salehi, 2007).

As CSF is in contact with the extracellular space of the brain, biochemical brain modifications could be reflected in the CSF and measurements of intrathecal peptides, growth factors, cytokines and amino acids might identify biomarkers of the meningitis. Researchers have sought to highlight the correlation between the meningitis process and potential biochemical markers in the CSF. Thus it is important to analyze CSF biochemistry to find a reliable biomarker. FGFs make up a large family of polypeptide growth factors that are found in organisms ranging from nematodes to humans. In vertebrates, the 22 members of FGF family range in molecular mass from 17 to 34 KDa and share 13-71 % amino acid identity. Between vertebrate species, FGFs have high conserved in both gene structure and amino acid sequence. FGFs have a high affinity for heparin sulfate proteoglycans and require heparin sulfate to activate one of four cell surface FGF receptors. During embryonic development, FGFs, play important roles in migration, proliferation and differentiation. In the adult organism, FGFs play important role in the tissue repair and response to injury. It is also important for neuronal signal transduction in both CNS and peripheral nervous system (PNS) (Ornitz and Itoh, 2001).

It has been demonstrated that exogenously applied FGF promotes regeneration of the CNS. FGF-1 induces the expression of NGF and is mitogenic for astrocytes in culture (Cassina et al., 2005). As the CSF is in contact with the extracellular space of the brain, biochemical brain abnormalities may be reflected in the CSF. I this study, the expression of FGF-1 in the CSF of patients with bacterial meningitis has been studied by Western blot. We studied FGF as it is an important growth factor in the CNS and a powerful mitogen (Zakrzewska et al., 2008).

2. Material and methods

2.1. Patients

After ethic committee's approval and informed consent the total of 33 Cerebrospinal fluid samples from normal control and children with bacterial meningitis were collected by lumbar puncture performed routinely on the basis of the clinical suspicion of neurological disease. Samples were aged matched between the two groups, analysed and ranged in age between 3 and 12 years. None of the patients suffered from known diabetes mellitus, earlier diagnosed tumors of the nervous system or infection. Samples were taken from both male and female patients. For the lumbar puncture the skin were cleaned with 70% alcohol. 0.2 ml of CSF were collected and used for this study. The samples that we used for analysis had no visible sign of contaminating neuroepithelium cells or red blood cells detectable under the microscope. When the tap was bloody samples were discarded. The samples were centrifuged at 10000 rpm for 5 minutes, the supernatant frozen immediately and stored at -70^{o}C until used. Thirty three samples from normal and patients with meningitis (n=33 for each group), were used for analysis of protein and NGF concentration. Three independent repeats of each analysis were carried out on each sample.

All values were expressed as mean±standard error of the mean (SEM). In all experiments, a minimum of 33 measurements were made in order to calculate a mean±SEM. Statistical analysis was performed using Student's t test and only values with $P \leq 0.05$ were considered statistically significant.

2.2. Protein analysis: Western blot

The total concentration of proteins in CSF was determined by the Bio-Rad protein assay based on the Bradford dye procedure. For Western blot analysis, CSF was mixed with a sample buffer containing 3.2% SDS, 15% glycerol, 2.8 M b-mercaptoeyhanol and 0.0015% bromophenol blue. Samples were applied to a 5–20% gradient SDS-PAGE gel (Bio-Rad, Milan, Italy) according to Laemly and the proteins obtained were transferred to nitrocellulose sheets, pore size 0.45 lm (Bio-Rad). After incubation for 2 hours at room temperature in the blocking solution (PBS containing 5% skimmed milk), the nitrocellulose sheets were exposed overnight, at 4 ^{o}C, to anti-FGF-1 monoclonal antibody (Abcam, Code 6201) and identified with a peroxidase-labeled mouse IgM PK 4010 Vectastain Avidin Biotin complex kit (Vectorlab, Peterborough, UK). The peroxidase activity was revealed with diaminobenzidine (0.5 mg/ml in PBS with 0.02% hydrogen peroxide).

3. Results

3.1. Total protein concentration

The total level of proteins in the CSF from patients with bacterial meningitis and controls was determined by the Bio-Rad protein assay. The total protein contents of CSF samples from patients with meningitis and the controls were 0.44 ± 0.06 and 0.43 ± 0.07 g/l, respectively. No significant difference has been seen in total protein concentration between two groups (P = 0.54).

3.2. Relative FGF-1 expression

We also analyzed CSF from controls and patients with meningitis using SDS-PAGE followed by silver staining. A difference between the gels was the presence of a low molecular weight protein in the CSF from patients with meningitis, which was weak in the CSF from normal. A Western blot analysis using anti-FGF-1 antibody as a probe confirmed the presence of FGF-1 (Figure 2A). In order to obtain semi-quantitative estimates of the relative amounts of 27 kDa protein, an image analyzer was used to determine the intensities of the band in the respective lanes. Quantification of the gels from repeated experiments (n=33) showed that the amount of FGF-1 was clearly increased in the CSF from patients with meningitis when compared with the normal CSF (Figure 2B). This study has shown that FGF-1 is present in human cerebrospinal fluid. The level of CSF FGF-1 in patients with meningitis is more than that in normal CSF.

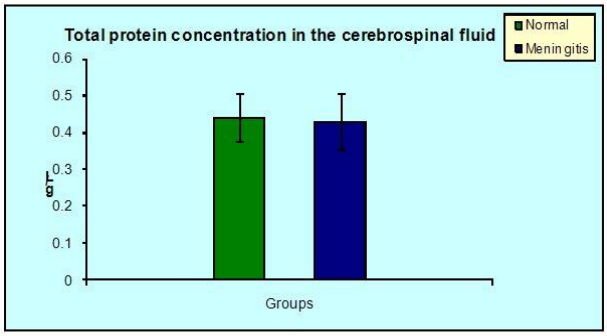

Figure 1. Total protein concentration (g/L) in the CSF (b) of normal control and patients with meningitis. No significant difference in CSF total protein concentrations was seen between the two groups (*P*= 0.54). In each experiment the total number of 33 samples were analysed.

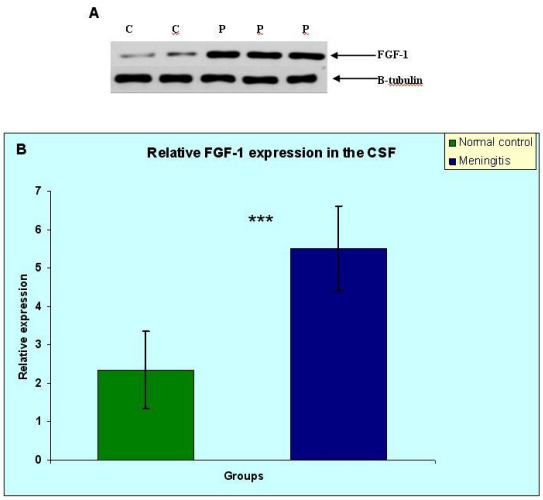

Figure 2. (A) Expression of FGF-1in the CSF samples from patients with meningitis and normal subjects. The arrows indicate the position of FGF-1 and β-tubulin (loading control). (B) Signal intensities from the anti-FGF-1 immunoblotting experiments A were determined by densitometric analysis using Metaview Software. Significance values are shown as stars: 3 stars P < 0.0001. In each experiment the total number of 33 samples were analysed. Abbreviations: C: Control; P: Patients with meningitis

4. Discussion

Meningitis remains an important cause of morbidity and mortality in childhood, even in the developed countries (Saez-Liorons, 1990). The examination of CSF is a keystone in the diagnostic procedure for patients with suspected meningitis. Diagnosis and initial treatment depends mainly on clinical symptoms and signs, as well as on the results of conventional CSF analysis, including number and type of cells and proteins (including growth factor and cytokines) levels; however, in some instances these clinical data do not allow differential diagnosis (Lindquist et al., 1988). Several biochemical markers have been studied with the aim of enabling accurate early diagnosis (Speer et al., 1988), but none of theses markers has satisfactory diagnostic value in this context.

In the initial stages of bacterial meningitis the release of inflammation mediators causes vasogenic brain edema with increased intracranial pressure (Ashwal et al., 1990). The present study indicates that there are elevated levels of FGF-1 in the CSF of patients with bacterial meningitis. Much research over the past two decades on the role of FGFs in growth, proliferation, aging and neurological disease has been demonstrated that FGF-1 is a powerful mitogen, exhibiting strong effects on numerous different cell types (Baird et al., 1986). FGF and its receptors are widely distributed in the nervous system. FGF protects against the degeneration of hippocampal neurons induced by brain ischemia. Endogenous FGF-1 is released into the CSF from the ependymal cells of the cerebral third ventricle (Sasaki et al., 1991). FGF-1 is important in neural cell survival (Vargas et al., 2006). After infarction of the lateral cerebral cortex in the rat brain, the level of FGF-1 increases during the first 3 weeks after stroke in tissues surrounding the infarct (Hara et al., 1994). As FGF-1 is involved in the regulation of neuron survival, it may have a role in the meningitis.

Glia and neurons have a major role in the synthesis of FGF-1 (Vargas et al., 2005). The increased level of FGF-1 in the CSF in the patients with bacterial meningitis in our stydy may be caused by increased generation of glial cells that resulted from brain damage. FGF-1 exerts its effects after CNS damage through apolipoprotein E secretion (Tada et al., 2004). Production of FGF-1 by glial cells in the brain of patients with meningitis represents an active response to neurological changes.

Understanding the signals that trigger neuronal proliferation and survival in the brain in vivo could assist the development of cell replacement therapy for neurological disorders such as meningitis. Efforts to identify these

signals have been aided by the ability to grow neuronal precursor cells in vitro. FGF-1 is important in neural cell survival and protects them from cell death (Hara et al., 1994). Considering the major roles of FGF-1 in the nervous system (Araki et al., 2007; Lin et al., 2009), it is possible that an increased expression of FGF-1 in the CSF of patients with bacterial meningitis may partially derived from peripheral circulation. The fact that there is a high penetration rate of injected protein into the brain supports this explanation. The comparison between plasma/serum and CSF levels should be included in the future studies to better delineate the site of FGF-1 production.

In summary, the expression of FGF-1 in the CSF is increased in the patients with meningitis, which suggests that it is involved in the pathophysiology of meningitis. These data supports the association between FGF-1 expression and meningitis. It is also concluded that CSF FGF-1 levels can be used in the early diagnosis of meningitis.

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References

Araki, M., Suzuki, H., Layer, P., 2007. Differential enhancement of neural and photoreceptor cell differentiation of cultured pineal cells by FGF-1, IGF-1, and EGF. Dev Neurobiol. 67, 1641-54.

Ashwal, S., Stringer, W., Tomasi, L., Schneider, S., Thompson, J., Perkin, R., 1990. Cerebral blood flow and carbon dioxide reactivity in children with bacterial meningitis. J Pediatr. 117, 523-30.

Baird, A., Mormède, P., Böhlen, P., 1986. Immunoreactive fibroblast growth factor (FGF) in a transplantable chondrosarcoma: inhibition of tumor growth by antibodies to FGF. J Cell Biochem. 30, 79-85.

Big, C., Reineck, L.A., Aronoff, D.M., 2009. Viral infections of the central nervous system: a case-based review. Clin Med Res. 7, 142-6.

Cassina, P., Pehar, M., Vargas, M.R., Castellanos, R., Barbeito, A.G., Estévez, A.G., Thompson, J.A., Beckman, J.S., Barbeito, L., 2005, Astrocyte activation by fibroblast growth factor-1 and motor neuron apoptosis: implications for amyotrophic lateral sclerosis. J Neurochem. 93, 38-46.

de Almeida, S.M., Nogueira, M.B., Raboni, S.M., Vidal, L.R., 2007. Laboratorial diagnosis of lymphocytic meningitis. Braz J Infect Dis. 11, 489-95.

Hara, Y., Tooyama, I., Yasuhara, O., Akiyama, H., McGeer, P.L., Handa, J., Kimura, H., 1994. Acidic fibroblast growth factor-like immunoreactivity in rat brain following cerebral infarction. Brain Res. 21, 101-7.

Leib, S.L., Tüber, M.G., 1999. [Meningitis (I)--differential diagnosis; aseptic and chronic meningitis]. Ther Umsch. 56, 631-9.

Lindquist, L., Linné, T., Hansson, L.O., Kalin, M., Axelsson, G., 1988. Value of cerebrospinal fluid analysis in the differential diagnosis of meningitis: a study in 710 patients with suspected central nervous system infection. Eur J Clin Microbiol Infect Dis. 7, 374-80.

Mashayekhi, F., Hadavi, M., Vaziri, H.R., Naji, M., 2010. Increased acidic fibroblast growth factor concentrations in the serum and cerebrospinal fluid of patients with Alzheimer's disease. J Clin Neurosci. 17, 357-9.

Mashayekhi, F., Salehi, Z., 2005. Expression of nerve growth factor in cerebrospinal fluid of congenital hydrocephalic and normal children. Eur J Neurol. 12, 632-7.

Mashayekhi, F., Salehin, Z., 2006. Cerebrospinal fluid nerve growth factor levels in patients with Alzheimer's disease. Ann Saudi Med. 26, 278-82.

Mashayekhi, F., Salehi, Z., 2007. Cerebrospinal fluid stem cell factor concentrations in the children with meningitis. Journal of Biological Sciences. 7, 1244-1248.

Ornitz, D.M., Itoh, N., 2001. Fibroblast growth factors. Genome Biol. 1, REVIEWS3005.

Sáez-Llorens, X., Ramilo, O., Mustafa, M.M., Mertsola, J., de Alba, C., Hansen, E., McCracken, G.H., 1990. Pentoxifylline modulates meningeal inflammation in experimental bacterial meningitis. Antimicrob Agents Chemother. 34, 837-43.

Sasaki, K., Oomura, Y., Suzuki, K., Muto, T., Hanai, K., Tooyama, I., Kimura, H., Yanaihara, N., 1991. Effects of fibroblast growth factors and platelet-derived growth factor on food intake in rats. Brain Res Bull. 27, 327-32.

Scheld, W.M., Quagliarello, V.J., Wispelwey, B., 1989. Potential role of host cytokines in Haemophilus influenzae lipopolysaccharide-induced blood-brain barrier permeability. Pediatr Infect Dis J. 8, 910-11.

Schlech, W.F., 3rd. 1992. The epidemiology of bacterial meningitis. Antibiot Chemother. 45, 5-17.

Sigurdardóttir, B., Björnsson, O.M., Jónsdóttir, K.E., Erlendsdóttir, H., Gudmundsson, S., 1997. Acute bacterial meningitis in adults. A 20-year overview. Arch Intern Med. 24, 425-30.

Speer, C.P., Rethwilm, M., Gahr, M., 1988. Elastase-alpha 1-proteinase inhibitor: an early indicator of septicemia and bacterial meningitis in childhood. Adv Exp Med Biol. 240, 485-91.

Tada, T., Ito, J., Asai, M., Yokoyama, S., 2004. Fibroblast growth factor 1 is produced prior to apolipoprotein E in the astrocytes after cryo-injury of mouse brain. Neurochem Int. 45, 23-30.

Thomas, K.E., Hasbun, R., Jekel, J., Quagliarello, V.J., 2002. The diagnostic accuracy of Kernig's sign,

Brudzinski's sign, and nuchal rigidity in adults with suspected meningitis. Clin Infect Dis. 1, 46-52.

Vargas, M.R., Pehar, M., Cassina, P., Beckman, J.S., Barbeito, L., 2006. Increased glutathione biosynthesis by Nrf2 activation in astrocytes prevents p75NTR-dependent motor neuron apoptosis. J Neurochem. 97, 687-96.

Vargas, M.R., Pehar, M., Cassina, P., Martínez-Palma, L., Thompson, J.A., Beckman, J.S., Barbeito, L., 2005. Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: consequences for motor neuron survival. J Biol Chem. 8, 25571-9.

Wilhelm, C., Ellner, J.J., 1986, Chronic meningitis. Neurol Clin. 4, 115-41.

Zakrzewska, M., Marcinkowska, E., Wiedlocha, A., 2008, FGF-1: from biology through engineering to potential medical applications. Crit Rev Clin Lab Sci. 45, 91-135.