

Cerebrospinal fluid hepatocyte growth factor level in meningitis

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Background and Purpose: Hepatocyte growth factor (HGF) is a multifunctional cytokine that has been found to be elevated in tuberculous and bacterial meningitis, but no evaluation has been undertaken of its usefulness in identifying various forms of aseptic meningitis.

Methods: In a retrospective study, the levels of HGF in the cerebrospinal fluid of 65 patients were measured prior to treatment. The association of HGF with non-infectious diseases and clinically or microbiologically proven bacterial, tuberculous, viral, fungal and parasitic meningitis was observed, along with its relation to other parameters of the cerebrospinal fluid.

Results: Forty six of the 65 patients (71%) were diagnosed as having meningitis. Cerebrospinal fluid HGF level was significantly elevated in patients with meningitis compared with patients with non-infectious diseases (1501 vs 578 pg/mL; Mann-Whitney *U* test, $p=0.001$). The highest HGF level was found in bacterial meningitis (2699 pg/mL), followed by tuberculous meningitis (1540 pg/mL), viral meningitis (1431 pg/mL), fungal meningitis (714 pg/mL) and parasitic meningitis (174 pg/mL). There was no association between HGF level and other parameters of the cerebrospinal fluid (Pearson's correlation test).

Conclusion: Cerebrospinal fluid HGF may offer additional information in the classification of meningitis. This may assist in patient management when no pathogen is cultured from the cerebrospinal fluid and when other parameters of the cerebrospinal fluid demonstrate equivocal results.

Key words: Cytokines; Diagnosis; Hepatocyte growth factor; Meningitis

Introduction

Hepatocyte growth factor (HGF) was initially extracted from rat platelets [1] and is initially found to be elevated in patients with hepatic diseases [2]. A multifunctional cytokine, it plays a crucial role in the recuperation of the liver after various forms of liver injuries such as acute or chronic hepatitis, liver failure and crush injuries, and is also elevated in liver metastasis, hepatocellular carcinoma and cholangiocarcinoma. HGF is also an organotrophic factor involved in regeneration of the kidney and the lungs after organ injury and is a neurotrophic factor in the brain and has prevented post-ischemic death of

neurons after occlusion of the middle cerebral artery [3]. It crosses the blood brain barrier through a saturable transport system [4], although an intrathecal production has also been postulated. An elevated level of HGF was reported in bacterial, viral and tuberculous meningitis, but an evaluation of this factor in other forms of aseptic meningitis has not been reported.

Methods

Patients

Sixty five patients were included in this retrospective study. All patients who presented at the emergency department of Kaohsiung Veterans General Hospital, a tertiary hospital in southern Taiwan, between October 2001 and April 2006, were eligible for inclusion. These patients were divided into 6 groups: group 1, patients

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with bacterial meningitis; group 2, patients with fungal meningitis; group 3, patients with parasitic meningitis; group 4, patients with viral meningitis; group 5, patients with tuberculous meningitis; and group 6, patients with medical conditions other than central nervous system infections.

Bacterial meningitis was defined as a clinical syndrome of meningitis with any of the following findings: classic cerebrospinal fluid (CSF) finding of bacterial meningitis such as a segmenter-predominant increase in white blood cell (WBC) count with an elevated protein, and low glucose level with a positive CSF Gram stain, culture, CSF bacterial antigen test [5] or blood cultures with no obvious source other than the central nervous system.

Fungal meningitis was characterized as meningitis with CSF abnormalities such as a lymphocytic pleocytosis with elevated protein, normal or depressed glucose level, with a positive India ink stain, positive CSF cryptococcal antigen or positive CSF culture [6].

Parasitic or helminthic meningitis is represented by a syndrome of headache, nuchal rigidity, fever, paresthesia and vomiting with a history of ingestion of raw mollusks. CSF findings demonstrate an increased CSF WBC count with an elevated protein and normal glucose level or a positive serologic study. CSF eosinophilia may not always be present [7]. Antibodies to *Angiostrongylus cantonensis* were detected in serum by a microenzyme-linked immunosorbent assay using young-adult worm antigen purified with a monoclonal antibody [8].

Viral meningitis was defined as meningitis with CSF findings compatible with viral meningitis, such as a lymphocyte-predominant increase in WBC count with a normal to slightly elevated protein, normal or depressed glucose with a positive CSF culture [9] or a clinical syndrome of viral infection, such as rhinorrhea, sore throat, or myalgia with or without a positive throat or rectal culture.

Tuberculous meningitis was defined as a syndrome of meningitis with a lymphocytic CSF pleocytosis with increased protein and low sugar level along with a positive CSF acid-fast stain or culture, or a radiographic finding compatible with pulmonary tuberculosis such as cavitations, and fibro-nodular infiltrates in the upper lung fields [10] with or without a positive sputum tuberculosis culture.

Determination of HGF

CSF samples obtained during consultation in the emergency department or immediately after admission

in the ward were thawed after being stored at -70°C . All specimens came from untreated patients and were centrifuged again prior to testing. HGF level was determined with a commercial kit (BioSource™ Hu HGF kit; Biosource International, Inc., Camarillo, CA, USA) utilizing a solid phase sandwich enzyme-linked immunosorbent assay. The assay was performed according to the manufacturer's instructions.

Statistical analysis

The difference between the HGF level of patients with meningitis and infectious diseases was analyzed by Mann-Whitney *U* test, while the difference of HGF levels among different forms of meningitis was analyzed by Kruskal-Wallis *H* test. Pearson correlation test was used to compare the differences of HGF levels and parameters of the CSF such as the WBC and red blood cell count, protein, lactate, and glucose level. A *p* value <0.05 was considered statistically significant.

Results

There was no statistically significant difference in demographic variables among the 6 groups, except that the group of patients with viral meningitis was relatively younger. A single CSF sample was evaluated for each of the 65 patients. Forty six patients had meningitis, while 19 patients with non-infectious diseases served as controls.

Total HGF levels ranged from 10 to 9268 pg/mL (mean, 1231 pg/mL) [Fig. 1]. Patients with meningitis had a mean HGF of 1501 pg/mL, which was significantly higher than the level in the CSF of patients with non-infectious diseases (578 pg/mL, Mann-Whitney *U* test; $p=0.001$) [Fig. 2]. The highest level was seen in patients with bacterial meningitis, followed by patients with tuberculous and viral meningitis (Table 1).

Patients with bacterial meningitis had HGF levels ranging from 10 to 9269 pg/mL, with a mean level of 2699 pg/mL. Among the 8 patients, one had a positive CSF Gram stain for Gram-negative bacilli and 3 had a positive CSF culture (*Klebsiella pneumoniae* was isolated in 1 patient and 2 patients grew *Streptococcus pneumoniae*). Three patients had positive blood cultures only, together with typical CSF findings for bacterial meningitis, with *Staphylococcus aureus*, *S. pneumoniae*, and *K. pneumoniae* isolated in different patients. One patient with *K. pneumoniae* liver abscess had a negative blood and CSF culture but had typical CSF findings for bacterial meningitis.

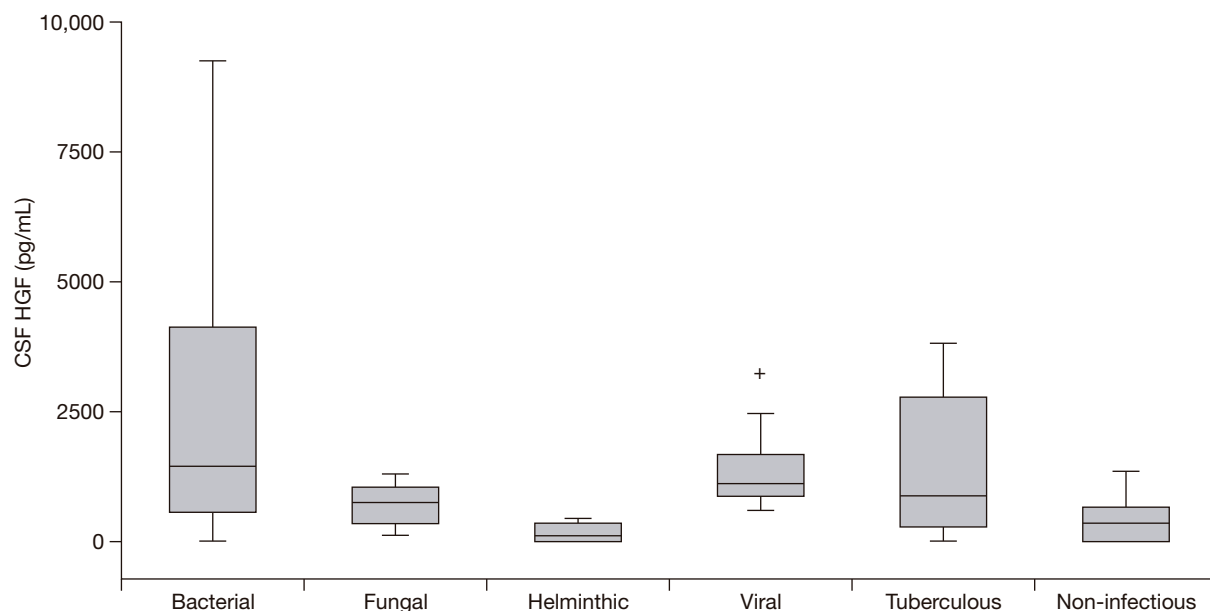


Fig. 1. Distribution of cerebrospinal fluid (CSF) hepatocyte growth factor (HGF) level in non-infectious diseases and different types of meningitis (Kruskal-Wallis H test).

All 4 cases of fungal meningitis were due to *Cryptococcus neoformans*, while all cases of helminthic meningitis were eosinophilic meningitis secondary to *Angiostrongylus cantonensis*. The CSF of 3 patients stained positive for India ink. Three patients had CSF cryptococcal antigen results ranging from 1:512 to 1:8192 and 1 patient had a negative cryptococcal antigen titer. All cases of eosinophilic meningitis had a

history of ingestion of mollusks and all had eosinophilic pleocytosis. Antibodies to *A. cantonensis* were detected in the serum of all patients.

Viral cultures were positive in 8 patients diagnosed with viral meningitis. Seven patients had a positive cerebrospinal culture: echovirus was isolated in 5 patients, while the 2 other patients had Coxsackie B5 and enterovirus isolated, respectively. The rest of the patients were diagnosed by a clinical syndrome of viral infection with CSF findings compatible with viral meningitis, with echovirus 6 being cultured from the rectum of 1 patient.

Patients with tuberculous meningitis had a mean HGF level of 1540 pg/mL. One patient with tuberculous meningitis grew *Mycobacterium tuberculosis* from the CSF. Eight other patients diagnosed due to a clinical syndrome of meningitis with a lymphocytic CSF pleocytosis with increased protein and low sugar level had radiographic findings compatible with pulmonary tuberculosis, with one patient having *M. tuberculosis* cultured from the sputum.

HGF levels in patients with aseptic meningitis were variable, with an elevated level seen in viral meningitis (1431 pg/mL) but not in fungal and helminthic meningitis (714 pg/mL and 174 pg/mL, respectively). There was no correlation between HGF levels and other parameters of the CSF such as the WBC and red blood cell count, and protein, lactate and glucose levels.

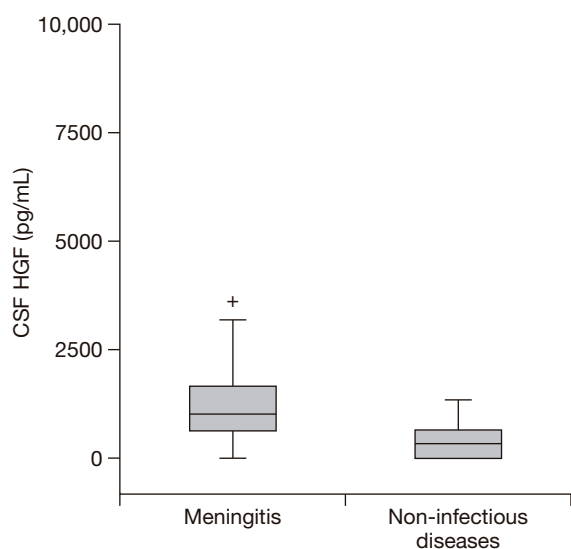


Fig. 2. Comparative level of cerebrospinal fluid (CSF) hepatocyte growth factor (HGF) in meningitis and non-infectious diseases (Pearson's correlation test).

Table 1. Demographic description and comparison of cerebrospinal fluid parameters in different etiologies of meningitis

Variable	Etiology of meningitis					
	Bacterial (n = 8)	Fungal (n = 4)	Helminthic (n = 4)	Viral (n = 21)	Tuberculous (n = 9)	Non-infectious (n = 19)
Gender (no.)						
Male	6	1	3	11	7	9
Female	2	3	1	10	2	10
Age (years)	22-80	31-64	32-73	0.07-74	34-89	0.08-88
[mean \pm SD]	(57.5 \pm 19.3)	(50.0 \pm 16.7)	(51.0 \pm 17.9)	(35.3 \pm 18.0)	(59.3 \pm 19.1)	(52.8 \pm 26.1)
WBC count (cells/ μ L; mean \pm SD) [no.]	1692 \pm 2272 (7)	135 \pm 128 (3)	572 \pm 218 (4)	177 \pm 181 (19)	131 \pm 120 (8)	2 \pm 1 (11)
RBC count (cells/ μ L; mean \pm SD) [no.]	678 \pm 706 (7)	810 \pm 910 (3)	28 \pm 38 (4)	3471 \pm 13,721 (19)	207 \pm 343 (8)	37 \pm 54 (11)
Protein (mg/dL; mean \pm SD) [no.]	470 \pm 401 (7)	119 \pm 34 (3)	150 \pm 74 (4)	97 \pm 50 (19)	150 \pm 66 (8)	56 \pm 22 (16)
Sugar (mg/dL; mean \pm SD) [no.]	41 \pm 27 (7)	26 \pm 23 (4)	41 \pm 2 (4)	59 \pm 17 (19)	53 \pm 24 (8)	85 \pm 28 (16)
HGF (pg/mL; mean \pm SD) [no.]	2699 \pm 3134 (8)	714 \pm 487 (4)	174 \pm 217 (4)	1431 \pm 818 (21)	1540 \pm 1516 (9)	578 \pm 927 (19)

Abbreviations: SD = standard deviation; WBC = white blood cell; RBC = red blood cell; HGF = hepatocyte growth factor

Discussion

The CSF level of HGF was significantly elevated in patients with meningitis compared to patients with non-infectious diseases. Early categorization of this disease prior to the culture results, which usually become available after a delay, would assist the clinician in the provision of proper treatment, a factor critical to the prognosis of the patient.

Since its isolation from rat platelets in the 1980s, HGF has been implicated in liver diseases [2], trophoblastic diseases [11], chronic renal failure [12], nephritis [13], leukemia [14], lung diseases [15], pancreatitis [16], gastric cancer [17], acute myocardial infarction [18] and peripheral arterial occlusive disease [19]. Di Renzo et al described its Met/HGF receptor in the central nervous system in 1993 [20] and association with diseases such as Alzheimer's disease, meningitis and glioma has been reported since [21-23]. HGF has been described as a neurotrophic factor and although previous reports have shown that infusion of HGF attenuates the neurological deficits seen after a central nervous system injury [24], no mortality benefit is seen in patients with elevated HGF levels.

The therapeutic role of HGF has been reported in spinal cord injury [25] and cerebral ischemia. Human recombinant HGF is reported to attenuate learning dysfunction attributed to cerebral ischemia, and reduce the disruption of the blood-brain barrier and the decrease in viable area of the brain after

ischemia [25]. Human recombinant HGF attenuated the decrease in the transmembrane protein occludin and membrane-associated guanylate kinase protein zonula occludens-1, which are components of the tight junctional barrier [26,27]. The attenuated decrease in these proteins may be a mechanism for the protective effect of human recombinant HGF for the protective effect against blood-brain barrier disruption [27]. A disruption of the blood-brain barrier is also described in meningitis, but the exact role of HGF in meningitis is not known. Nayeri et al observed a significantly elevated level of HGF in the CSF of patients with bacterial meningitis in comparison to patients with Lyme disease, viral meningitis, HSV encephalitis and facial palsy [23]. Other investigators observed a similarly elevated titer in bacterial meningitis. Ozden et al found that this level is lower than the titer in patients with tuberculous meningitis [28], while Kern et al showed no significant elevation of HGF in patients with aseptic meningitis [29].

Our study showed significantly elevated HGF level in patients with bacterial, tuberculous and viral meningitis with no increase in level observed in cryptococcal and eosinophilic meningitis. This is inconsistent with the result of Kern et al [29] since, while an elevation of this cytokine may not discriminate viral from bacterial meningitis, it may serve as a marker to differentiate viral meningitis from other forms of aseptic meningitis. The reason for a non-elevated level in cryptococcal and eosinophilic meningitis is not known.

Early diagnosis is a crucial factor in the prognosis of patients with meningitis. Assay of HGF in the CSF may assist in the management of patients when no pathogen is cultured from the CSF and when other parameters of the CSF demonstrate equivocal results. This study demonstrates that HGF levels may be useful in discriminating bacterial, tuberculous and viral meningitis from non-infectious diseases and other forms of aseptic meningitis.

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