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ORIGINAL ARTICLE

Cerebrospinal fluid vitamin D-binding protein as a new biomarker for the diagnosis of meningitis

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Abstract

Background Meningitis is an inflammatory process involving meninges. It is difficult to diagnose because of the absence of a diagnostic biomarker. We first report here the possibility of cerebrospinal fluid (CSF) vitamin D-binding protein (VDBP) as a new biomarker for the diagnosis of meningitis.

Methods This prospective study enrolled a total of 102 subjects (58 patients with non-neurologic disease, 17 patients with meningitis, and 27 patients with other neurologic diseases) from 2017 to 2018. CSF and blood samples were collected in pairs. Total 25(OH)D in CSF and serum and VDBP levels in serum were measured. GC genotyping was also performed to determine polymorphisms of rs4588 and rs7041. CSF total 25(OH)D and VDBP levels were compared with serum total 25(OH)D and VDBP levels according to disease (meningitis vs. non-meningitis). Receiver operating characteristic (ROC) analysis for the diagnosis of meningitis using CSF VDBP level was performed.

Results Mean CSF VDBP and serum VDBP levels of all patients were 1.48 ± 1.32 and 181.28 ± 56.90 μg/mL, respectively. CSF VDBP level in the meningitis disease group (3.20 ± 1.49 μg/mL) was significantly ($P < 0.001$) higher than that in other disease groups. According to ROC curve analysis, the appropriate cut-off value for CSF VDBP was 1.96 μg/mL, showing sensitivity of 82.4% and specificity of 85.9%. AUC of CSF VDBP was 0.879 (95% CI: 0.789–0.962).

Conclusions CSF VDBP level showed very good diagnostic performance. It could be used as a potential biomarker for the diagnosis of meningitis.

Keywords Cerebrospinal fluid · Vitamin D-binding protein · Meningitis · Biomarker

Introduction

Meningitis is an inflammatory process involving meninges. Aseptic meningitis is the most common form. The annual incidence of meningitis is unknown because of underreporting. However, European studies have reported 70 cases per 100,000 children younger than 1 year, 5.2 cases per 100,000 children 1 to 14 years of age, and 7.6 per 100,000 adults [1, 2]. Aseptic is differentiated from bacterial meningitis if there is meningeal inflammation without signs of bacterial growth in cultures. Bacterial meningitis is an infectious disease of the central nervous system (CNS) characterized by significant mortality and morbidity despite advances in antibiotics [3]. The mortality of bacterial meningitis varies from 3 to 21%, depending on the type of organism [4]. Furthermore, survivors of bacterial meningitis have a high risk of having cognitive impairment or other neurological deficits [5, 6].

Because of poor performance of clinical signs to rule out meningitis, all patients who present with symptoms of meningitis should undergo prompt lumbar puncture (LP) and evaluation of cerebrospinal fluid (CSF) for definitive diagnosis. CSF findings typical of aseptic meningitis include relatively

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low and predominantly lymphocytic pleocytosis, normal glucose level, and normal to slightly elevated protein level. Bacterial meningitis classically has very high and predominantly neutrophilic pleocytosis, low glucose level, and high protein level [7]. However, this is not the case for all patients. It can vary in older patients and those who have partially treated meningitis or immunosuppression [8]. Differential cell count of CSF can also vary depending on proficiency of the examiner. Thus, additional new biomarkers are needed to have more accurate diagnosis of meningitis.

Vitamin D-binding protein (VDBP) is a 58-kDa multifunctional protein produced in the liver that circulates in the plasma. VDBP is an acute phase reactant. Thus, its level can change depending on various conditions [9–12]. VDBP was initially named group-specific component globulin. It is known to play a major role in vitamin D metabolic transport. However, other various roles of VDBP have been recently reported, including actin sequestration and regulation of immune responses [13].

The gene encoding VDBP (*GC*) has a high rate of polymorphism. The frequency of its genotype is different depending on ethnic population. In addition, differences in VDBP affinity for 25(OH)D depending on its genotype have been reported. Two single-nucleotide polymorphisms (SNPs), rs7041 and rs4588, give rise to three major polymorphic isoforms of VDBP (GC1F, GC1S, and GC2). Their frequencies also differ among ethnic populations. The isoform GC1F has the highest affinity for vitamin D, followed by GC1S and GC2 [14, 15].

The presence of VDBP has been demonstrated in serum, urine, breast milk, ascitic fluid, cerebrospinal fluid, saliva, seminal fluid, and surfaces of lymphocytes, neutrophils, and monocytes. Differential mRNA expression of VDBP has been reported in brain, heart, lungs, kidneys, placenta, spleen, testes, and uterus [13, 16]. In comparison with blood which has the highest concentration of VDBP, lower expression levels of VDBP have been detected in other body fluids [17]. Although VDBP has been found in human CSF [18], it is unclear if it is synthesized in the CNS. Recently, it has been reported that regions of supraoptic and paraventricular neurons exhibit VDBP immunoreactivity in the animal experiment [19]. Previous studies have reported that changes in VDBP concentration are linked to the pathology of various diseases including multiple sclerosis [13, 17, 20]. In addition, Gressner et al. [21] have reported that intrathecal synthesis of VDBP is increased in patients with severe neurodegeneration including AD, suggesting that upregulated VDBP may act as an actin scavenger in neurodegenerative diseases. However, no previous study has reported VDBP level in CSF of patients with meningitis. Thus, the objective of this study was to evaluate the performance of CSF VDBP as a new biomarker of for the diagnosis of meningitis. We first report here that the VDBP is a candidate for a new biomarker which needs further evaluation.

Materials and methods

Study subjects

This prospective study enrolled a total of 102 subjects who underwent lumbar puncture for the purpose of diagnosis from September 2017 to May 2018. Patients' CSF and blood samples were collected in pairs on the same day. We collected clinical and laboratory data including age, sex, cellular differential count of CSF, chemical analysis of CSF including glucose and protein level, and final diagnosis from electronic medical records.

These enrolled patients were classified into three groups (non-neurologic disease, meningitis, and other neurologic disease) depending on results of CSF analysis and the final diagnosis. If the result of CSF analysis was normal and the final diagnosis was not related to neurologic pathology, patients were classified as having non-neurologic disease. We classified patients as meningitis if either results of CSF analysis or clinical symptom strongly suggested meningitis. If the result of CSF analysis was normal while the final diagnosis suggested other neurologic pathology, then patients were classified as having other neurologic diseases. When the number of red blood cells exceeded 1000 in differential count for CSF, it was regarded as traumatic tapping and excluded from this study.

At the time of study enrollment, CSF and blood samples were collected. Serum and leukocytes were separated and stored at − 80 °C. The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (approval number: 2017-03-010). Written informed consent was obtained from all participants.

VDBP and total 25(OH)D assays

For CSF and serum samples, VDBP level and total 25(OH)D level were measured. VDBP level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Total 25(OH)D level was measured using an Elecsys Vitamin D Total electrochemiluminescence binding assay (Roche Diagnostics, Mannheim, Germany) and a Cobas 8000 e602 analyzer (Roche Diagnostics).

GC *genotyping*

Genomic DNA was isolated from peripheral blood leukocytes using a DNeasy Blood and Tissue Kit (Qiagen,

Hilden, Germany) according to the manufacturer's instructions. *GC* genotyping for rs7041 and rs4588 was performed using a TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA, USA) and an ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to manufacturers' instructions. Common *GC* alleles were determined as follows: Gc1f (c.1296T; c.1307C), Gc1s (c.1296G; c.1307C), and Gc2 (c.1296T; c.1307A).

Statistical analysis

Frequencies and percentage (%) are calculated for categorical variables while median value and range are presented for continuous variables. We compared vitamin D-related data and *GC* genotypes between groups using Pearson's Chi-square test for categorical variables. The significance of normally distributed variables was determined by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. The relationship between CSF VDBP and serum VDBP was evaluated by simple correlation analysis. ROC curve and area under the ROC curve (AUC) were used to evaluate the performance of CSF VDBP. Reference interval was calculated according to guidelines of the Clinical and Laboratory Standards Institute. After excluding outliers using Tukey method, data set was demonstrated using nonparametric analysis (2.5–97.5th percentile interval). All statistical analyses were performed using PAWS Statistics software, version 18.0 (SPSS Inc., Chicago, IL, USA) and MedCalc Statistical software, version 17.2 (Mariakerke, Belgium). P values < 0.05 were considered statistically significant.

Results

General characteristics of study subjects

General characteristics of study subjects are shown in Table 1. One hundred and two patients were enrolled in this study. Male and female ratio was 0.79:1. Median age of study subjects was 50.5 years. Among these 102 patients, 58 (56.9%), 17 (16.7%), and 27 (26.4%) patients were classified into non-neurologic disease, meningitis, and other neurologic disease groups, respectively. In the meningitis group, 3 (17.6%), 13 (76.5%), and 1 (5.9%) patients had bacterial meningitis, viral meningitis, and tuberculosis meningitis, respectively. In the other neurologic disease group, patients had tumors, motor neuron disease, seizure, hydrocephalus, and others.

Table 1 Patients' characteristics

Characteristics	Result
Total number of patients	102
Sex ratio (male/female)	0.79:1 (45:57)
Age, median (IQR)	50.5 (11.5–66)
Age, number of cases	
< 1 year	15
1~17 years	16
18~65 years	44
> 65 years	27
Disease, number of cases	
Non-neurologic disease	58
Meningitis	17
Bacterial meningitis	3
Viral meningitis	13
Tuberculosis meningitis	1
Other neurologic disease	27
Tumor	7
Motor neuron disease	5
Seizure	5
Hydrocephalus	3
Encephalopathy	2
Plexopathy	2
Decreased mentality	1
Moyamoya syndrome	1
Parkinson syndrome	1

IQR interquartile range

Comparison of vitamin D-binding protein (VDBP) and total 25(OH)D levels in CSF and serum according to patients' disease status

Table 2 shows mean ± SD of vitamin D variables according to patients' disease status. Mean total 25(OH)D concentrations in CSF and serum of all patients were 37.42 ± 7.58 and 26.29 ± 12.84 ng/mL, respectively. Mean CSF VDBP and serum VDBP of all patients were 1.48 ± 1.32 and 181.28 ± 56.90 μg/mL, respectively. Total 25(OH)D in CSF was 1.42 times higher than that in the serum. The mean serum VDBP level was approximately 122 times higher than the mean CSF VDBP level in all study subjects. CSF VDBP level in the meningitis group (3.20 ± 1.49 μg/mL) was significantly ($P < 0.001$) higher than that in the other two groups. CSF VDBP levels of viral meningitis, bacterial meningitis, and tuberculosis meningitis were 3.47 ± 1.43, 1.87 ± 1.52, and 3.76, respectively. However, there were no significant differences in VDBP levels among the subgroups of meningitis ($P = 0.105$). CSF total 25(OH)D, serum total 25(OH)D, and serum VDBP concentrations were not significantly different among

Table 2 Comparison of vitamin D and VDBP levels in serum and CSF by disease

Disease group	CSF total 25(OH)D (ng/mL)	CSF VDBP (μg/mL)	Serum total 25(OH)D (ng/mL)	Serum VDBP (μg/mL)
Non-neurologic disease	37.78 ± 7.75	0.91 ± 0.71	27.37 ± 14.05	177.17 ± 58.51
Meningitis	37.94 ± 7.46	3.20 ± 1.49	24.79 ± 11.40	199.29 ± 35.81
Viral meningitis	*36.85 ± 8.21*	*3.47 ± 1.43*	*22.65 ± 11.55*	*199.64 ± 37.72*
Bacterial meningitis	*41.39 ± 2.83*	*1.87 ± 1.52*	*28.90 ± 8.05*	*195.78 ± 41.06*
Tuberculosis meningitis	*41.64*	*3.76*	*40.27*	*205.26*
Other neurologic disease	36.34 ± 7.44	1.62 ± 1.29	24.91 ± 10.99	178.78 ± 63.47
Total	37.42 ± 7.58	1.48 ± 1.32	26.29 ± 12.84	181.28 ± 56.90
P value*	0.69	*< 0.001*	0.623	0.361

Data are expressed as means ± SD

CSF cerebrospinal fluid, *VDBP* vitamin D-binding protein, *25(OH)D* 25-hydroxyvitamin D

**P* value was calculated with non-neurologic disease, meningitis, and other neurologic disease

disease groups. We also obtained box plot graph of CSF VDBP concentrations in the three disease groups (Fig. 1).

Distribution of vitamin D-binding protein genotypes and comparison of vitamin D and VDBP levels in serum and CSF by genotypes

Gc1f-1s ($n = 38$, 37.3%) was the most common genotype, followed by Gc2-1f type ($n = 18$, 17.6%) and Gc1f-1f type ($n = 16$, 15.7%) in total study patients. The most frequent three genotypes were in the order of Gc1f-1s > Gc2-1f > Gc1f-1f in the non-neurologic disease group, Gc1f-1s > Gc1s-2 > Gc2-1f in the meningitis group, and Gc1f-1s > Gc1f-1f > Gc1s-2 in other neurologic disease group. There was no significant difference in genotype distribution among the three groups ($p = 0.374$, Table 3). CSF total 25(OH)D and CSF VDBP concentrations were the highest in those with Gc1s-1s genotype, but the lowest in CSF total 25(OH)D with Gc1s-2 genotype and CSF VDBP with Gc 1f-1f genotype. Serum total 25(OH)D and serum VDBP concentrations were the highest in those with Gc1s-2 genotype. However, there were no significant differences in CSF/serum total 25(OH)D or VDBP level according to VDBP genotype (Table 4).

Receiver operating characteristic analysis for the diagnosis of meningitis using CSF VDBP level

We calculated the optimal cut-off value of CSF VDBP for detecting meningitis from ROC curve analysis. According to ROC curve analysis, the appropriate cut-off value of CSF VDBP for the diagnosis of meningitis was 1.96 μg/mL with sensitivity of 82.4% and specificity of 85.9%. AUC of CSF VDBP was 0.879 (95% CI: 0.789–0.962) (Fig. 2).

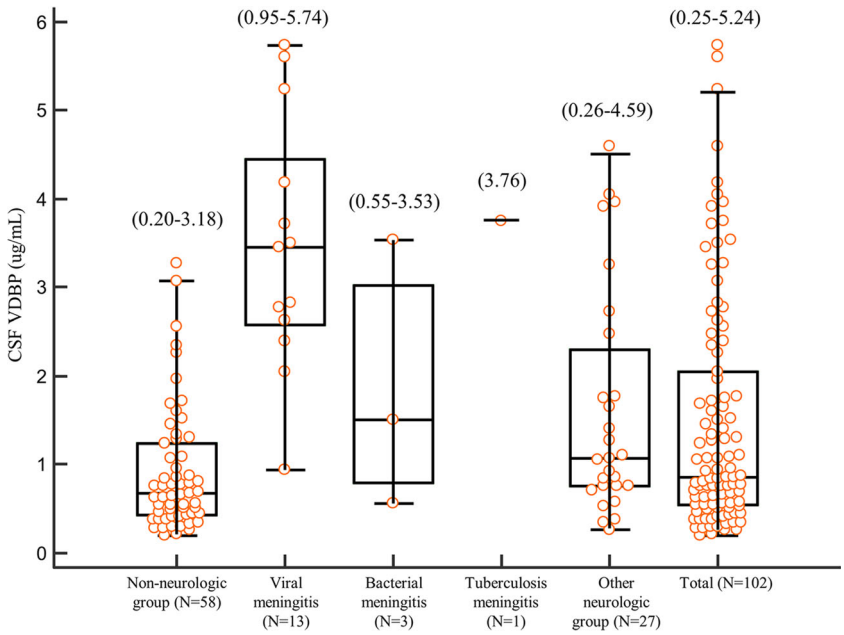

Fig. 1 VDBP level in meningitis and non-meningitis patients. The central box represents values from the lower to the upper quartile. The middle line represents the median. Bars represent 2.5th and 97.5th percentiles

Table 3 Distribution of vitamin D-binding protein genotypes according to disease

Genotype group	Gc1f-1f	Gc1f-1s	Gc1s-1s	Gc2-1f	Gc1s-2	Gc2-2
Non-neurologic disease	11	17	5	13	5	7
Meningitis	1	6	1	3	4	2
Other neurologic disease	4	15	1	2	3	2
Total	16	38	7	18	12	11

Discussion

VDBP is a protein which has many functions such as transport of vitamin D metabolites, actin sequestration, and regulation of immune responses [13]. VDBP is mainly synthesized in hepatic parenchymal cells, although it is also synthesized in kidneys, testis, yolk sac, and abdominal fat [18]. In case of animals, it has been confirmed that VDBP is synthesized in rat hypothalamus [22]. VDBP is also present in human CSF, although it is currently unclear whether VDBP is synthesized in the CSF. In addition, CSF VDBP has been studied in some neurologic diseases such as multiple sclerosis [22]. However, it has never been studied in meningitis. To the best of our knowledge, this is the first study to investigate VDBP levels in patients with meningitis.

In this study, we demonstrated that CSF VDBP levels were significantly ($p < 0.001$) increased in patients with meningitis compared to those in patients without meningitis. Furthermore, ROC curve analysis showed a good predictive value for CSF VDBP levels in meningitis patients. At a cut-off value of 1.96 μg/mL, CSF VDBP for the diagnosis of meningitis showed a sensitivity of 82.4% and a specificity of 85.9%. This finding implies that CSF VDBP level could be a useful new biomarker to diagnose meningitis. Interestingly, all CSF samples were collected from patients within 1~2 days after symptoms developed. Thus, it could be inferred that CSF VDBP started to increase in the very early phase of meningitis. Additional investigation is needed to determine when CSF VDBP begins to increase in meningitis. If it is increased early, it could be used as a good biomarker for early diagnosis of meningitis which is very useful clinically.

Up to date, very few studies have reported CSF-related biomarkers to diagnose meningitis. Savonius et al. [23] have measured CSF cathelicidin in childhood bacterial meningitis at 12–24-h intervals and compared serum 25 (OH) D values, without finding a significant correlation. Another study has shown that CSF procalcitonin concentration > 0.085 ng/mL appears to be a reliable indicator of bacterial meningitis with sensitivity of 55.17% and specificity of 95.83% [24]. CSF VDBP levels in our study showed very good diagnostic performance with AUC of 0.875 compared to results of previous studies. In fact, few previous studies have detected VDBP levels in CSF. CSF VDBP concentrations were measured by qualitative or semi-quantitative methods in most of previous studies by using electrophoresis method [22, 25]. However, in our study, CSF VDBP concentrations were quantitatively measured using ELISA method which could result in more reliable quantitative results.

In our study, the mean serum VDBP level was approximately 122 times higher than the mean CSF VDBP level in all study subjects. There was no significant correlation between CSF and serum VDBP levels (Fig. 3). These

Table 4 Comparison of vitamin D levels in serum and CSF by genotypes

Genotype group	CSF total 25(OH)D (ng/mL)	CSF VDBP (μg/mL)	Serum total 25(OH)D (ng/mL)	Serum VDBP (μg/mL)
Gc1f-1f ($N = 16$)	36.57 ± 8.84	0.89 ± 0.50	23.31 ± 12.16	184.36 ± 74.37
Gc1s-1f ($N = 38$)	36.56 ± 8.31	1.77 ± 1.60	26.54 ± 11.91	187.90 ± 56.29
Gc1s-1s ($N = 7$)	40.73 ± 2.47	1.90 ± 1.46	24.45 ± 14.11	180.44 ± 47.78
Gc2-1f ($N = 18$)	38.45 ± 7.33	1.20 ± 1.27	27.49 ± 13.51	162.34 ± 50.51
Gc2-1s ($N = 12$)	36.16 ± 8.76	1.51 ± 1.19	33.55 ± 14.81	190.70 ± 59.51
Gc2-2 ($N = 11$)	39.25 ± 2.92	1.52 ± 1.07	21.05 ± 11.54	175.18 ± 45.61
Total ($N = 102$)	37.42 ± 7.58	1.48 ± 1.32	26.29 ± 12.84	181.28 ± 56.90
P value	0.373	0.232	0.282	0.445

Data are expressed as means ± SD

CSF cerebrospinal fluid, *VDBP* vitamin D-binding protein, *25(OH)D* 25-hydroxyvitamin D

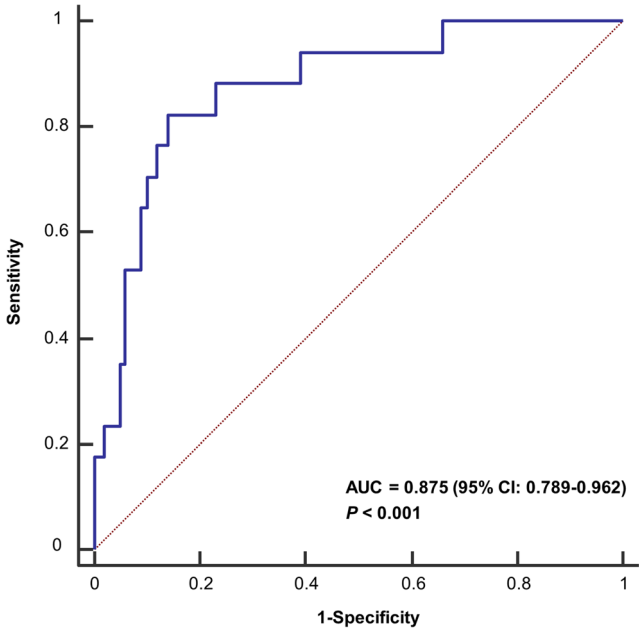

Fig. 2 Receiver operating characteristic (ROC) curve analysis of VDBP level in CSF for diagnosis of meningitis

results are in line with a previous study showing that the transition of VDBP from serum to CSF via blood–brain barrier (BBB) is very limited [26]. In addition, our study showed that serum and CSF VDBP concentrations were higher in the meningitis group than those in the other two groups. In addition, there were significant differences in CSF VDBP levels among disease groups. Blood proteins are largely excluded from CSF by the BBB [27]. Inflammation is intrinsically linked to mechanisms of tight junction deregulation which contributes to loss of BBB function in the CNS [28]. In case of CNS infections, elevated CSF protein concentrations usually result from diffusion of blood proteins as a consequence of disruption of tight junctions between endothelial cells of venules and other small meningeal vessels [27]. Results of the present study suggest that high CSF VDBP levels in meningitis might be due to such mechanism. Although not yet clarified, if VDBP is synthesized in the brain, the synthesis of VDBP in the brain might have been increased in order to control inflammatory reactions such as meningitis. Additional investigation is needed to test this hypothesis.

In this study, the mean CSF total 25(OH)D level was generally 1.4-fold higher than serum total 25(OH)D level. This

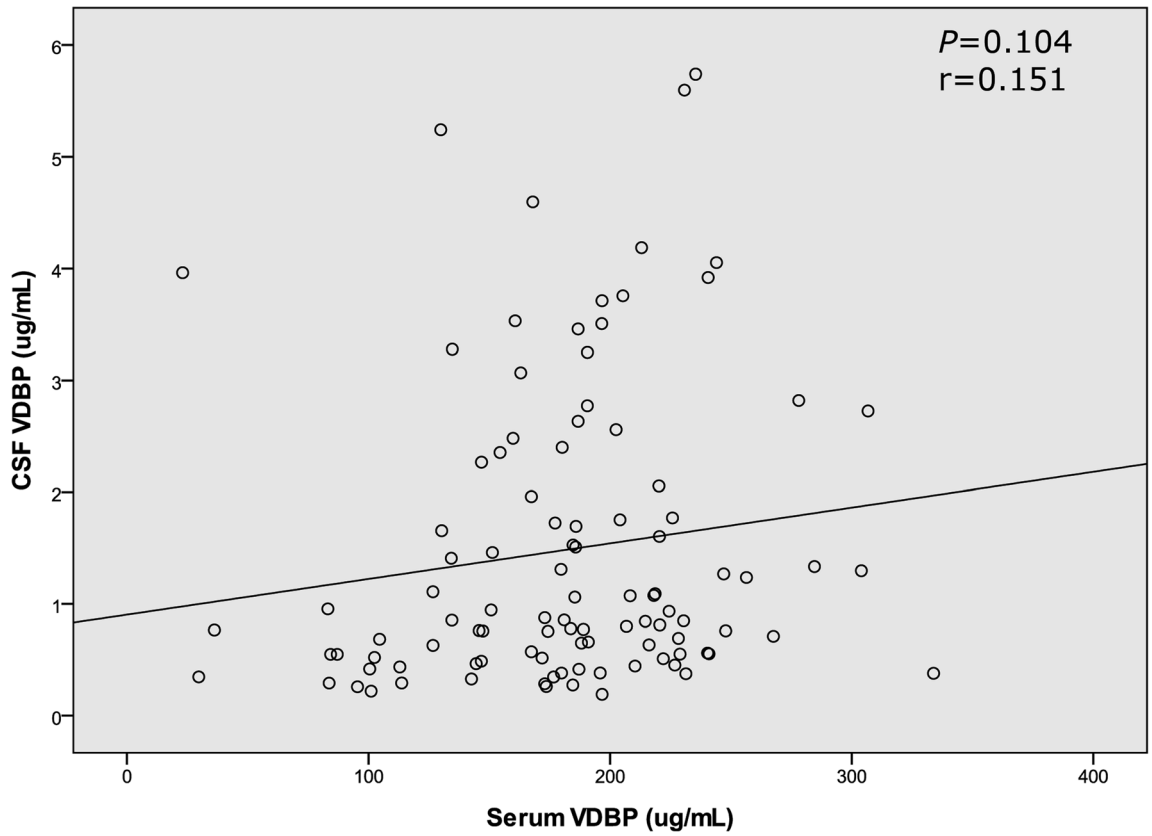

Fig. 3 Correlation analysis of CSF and serum VDBP concentrations (r = Pearson correlation coefficient)

relatively higher concentration of vitamin D in the brain may support the possible role of vitamin D in modulating inflammation and inducing regeneration of the neuron suggested in a previous study [29]. In addition, our finding may imply that there is a special unidirectional transport system from general circulation to brain in BBB to maintain higher intrathecal vitamin D status. Most (85–90%) of the circulating 25(OH)D in the serum are tightly bound to 58-kDa-sized vitamin D-binding protein (VDBP), with a smaller amount (10–15%) loosely bound to albumin. Only less than 1% of circulating vitamin D exists in free unbound form [30–32]. It has been reported that VDBP-bound 25(OH)D, the most abundant form of vitamin D in serum, can be transported by megalin/cubilin transport system, the only transport system found for VDBP-bound vitamin D [33]. Thus, this megalin/cubilin transport system might exist in BBB. To test this hypothesis, further studies including animal model investigations are needed.

The major *GC* genotype and allele frequencies are known to vary among ethnicities [15]. For example, Nielson et al. have reported that nearly all African American subjects and all Gambian subjects have the Gc1f allele (Gc1f-Gc1f, Gc1f-Gc1s, or Gc1f-Gc2). In contrast, most white subjects do not have the Gc1f allele while Gc1s-Gc1s and Gc1s-Gc2 are the most frequent genotypes in this group [34]. Koreans have different GC allele frequencies from African Americans and whites. Jung et al. [35] have enrolled 203 patients with chronic obstructive pulmonary disease and 157 control subjects and reported that Gc1f-Gc2 (25%) is the most frequent genotype, followed by Gc1f-Gc1f (22%), Gc1f-Gc1s (20%), and Gc1s-Gc2 (18%). Although their results are not exactly the same as our results, they are similar in that the three most frequent genotypes have Gc1f allele. Such difference in the distribution of Gc genotype could be explained by the relatively small number of study subjects enrolled in our study.

Recently, studies on VDBP polymorphism related to various diseases have been steadily researched. In a previous study, VDBP Gc1f-1f genotype was positively associated with the risk of acute myocardial infarction in subject over 45 years after adjusting for potential confounders [36]. Another study has shown that women with vitamin D deficiency (< 20 ng/mL), GT allele of VDBP SNP rs7041, and VDBP allelic combination Gc1f-1f are associated with increased risk of developing polycystic ovarian syndrome [37]. In our study, only one patient of Gc1f-1f genotype was present in the meningitis group. It was present at a lower rate than that in other groups. Since the number of cases in this study was very small, we could not find any significant difference between meningitis and VDBP genotype.

This study has some limitations. First, this was a cross-sectional study. Second, although the disease group included various diseases, the number of patients included in each group was relatively small. Further research with larger number of patients for each group would be necessary. Third, we did not use liquid chromatography-tandem mass spectrometry (LC-MS/MS), a gold standard method, to measure total 25(OH)D levels. However, the Elecsys Vitamin D Total Kit with the Cobas e602 module (Roche Diagnostics, Mannheim, Germany), the assay used for this study, has been previously found to be an assay comparable to LC-MS/MS method [38].

Conclusions

In this study, we observed a significant increase of CSF VDBP level in Korean patients with meningitis. In addition, we suggested an optimal cut-off value for CSF VDBP level to diagnose meningitis. In ROC analysis, CSF VDBP level at a cut-off value of 1.96 μg/mL showed very good diagnostic performance, with sensitivity of 82.4%, specificity of 85.9%, and AUC of 0.875. While we also demonstrated the distribution of VDBP genotype, there was no significant relation between VDBP genotype and meningitis. Thus, CSF VDBP could be a potential biomarker for the diagnosis of meningitis.

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Compliance with ethical standards The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (approval number: 2017-03-010). Written informed consent was obtained from all participants.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, Harrison LH, Farley MM, Reingold A, Bennett NM, Craig AS, Schaffner W, Thomas A, Lewis MM, Scallan E, Schuchat A, Emerging Infections Programs N (2011) Bacterial meningitis in the United States, 1998-2007. N Engl J Med 364(21):2016–2025. https://doi.org/10.1056/NEJMoa1005384
2. Martin NG, Iro MA, Sadarangani M, Goldacre R, Pollard AJ, Goldacre MJ (2016) Hospital admissions for viral meningitis in children in England over five decades: a population-based observational study. Lancet Infect Dis 16(11):1279–1287. https://doi.org/10.1016/S1473-3099(16)30201-8
3. van de Beek D, de Gans J, Tunkel AR, Wijdicks EF (2006) Community-acquired bacterial meningitis in adults. N Engl J Med 354(1):44–53. https://doi.org/10.1056/NEJMra052116
4. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, Lefkowitz L, Perkins BA (1997) Bacterial meningitis in the United States in 1995. Active surveillance team. N Engl J

Med 337(14):970–976. https://doi.org/10.1056/NEJM199710023371404
5. Hoogman M, van de Beek D, Weisfelt M, de Gans J, Schmand B (2007) Cognitive outcome in adults after bacterial meningitis. J Neurol Neurosurg Psychiatry 78(10):1092–1096. https://doi.org/10.1136/jnnp.2006.110023
6. van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M (2004) Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med 351(18):1849–1859. https://doi.org/10.1056/NEJMoa040845
7. Mount HR, Boyle SD (2017) Aseptic and bacterial meningitis: evaluation, treatment, and prevention. Am Fam Physician 96(5):314–322
8. Weisfelt M, van de Beek D, Spanjaard L, Reitsma JB, de Gans J (2006) Community-acquired bacterial meningitis in older people. J Am Geriatr Soc 54(10):1500–1507. https://doi.org/10.1111/j.1532-5415.2006.00878.x
9. Bhan I (2014) Vitamin d binding protein and bone health. Int J Endocrinol 2014:561214. https://doi.org/10.1155/2014/561214
10. Chun RF (2012) New perspectives on the vitamin D binding protein. Cell Biochem Funct 30(6):445–456. https://doi.org/10.1002/cbf.2835
11. Heijboer AC, Blankenstein MA, Kema IP, Buijs MM (2012) Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 58(3):543–548. https://doi.org/10.1373/clinchem.2011.176545
12. Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, Terrault NA, Stotland N, Bikle D (2014) A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations. J Clin Endocrinol Metab 99(5):1631–1637. https://doi.org/10.1210/jc.2013-3874
13. Gomme PT, Bertolini J (2004) Therapeutic potential of vitamin D-binding protein. Trends Biotechnol 22(7):340–345. https://doi.org/10.1016/j.tibtech.2004.05.001
14. Arnaud J, Constans J (1993) Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 92(2):183–188
15. Kamboh MI, Ferrell RE (1986) Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. Hum Genet 72(4):281–293
16. Cooke NE, McLeod JF, Wang XK, Ray K (1991) Vitamin D binding protein: genomic structure, functional domains, and mRNA expression in tissues. J Steroid Biochem Mol Biol 40(4–6):787–793
17. Meier U, Gressner O, Lammert F, Gressner AM (2006) Gc-globulin: roles in response to injury. Clin Chem 52(7):1247–1253. https://doi.org/10.1373/clinchem.2005.065680
18. White P, Cooke N (2000) The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab 11(8):320–327
19. Jirikowski GF, Kaunzner UW, Dief Ael E, Caldwell JD (2009) Distribution of vitamin D binding protein expressing neurons in the rat hypothalamus. Histochem Cell Biol 131(3):365–370. https://doi.org/10.1007/s00418-008-0540-6
20. Speeckaert M, Huang G, Delanghe JR, Taes YE (2006) Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 372(1–2):33–42. https://doi.org/10.1016/j.cca.2006.03.011
21. Gressner OA, Schifflers MC, Kim P, Heuts L, Lahme B, Gressner AM (2009) Questioning the role of actinfree Gc-globulin as actin scavenger in neurodegenerative central nervous system disease: relationship to S-100B levels and blood-brain barrier function. Clin Chim Acta 400(1–2):86–90. https://doi.org/10.1016/j.cca.2008.10.015
22. Yang M, Qin Z, Zhu Y, Li Y, Qin Y, Jing Y, Liu S (2013) Vitamin D-binding protein in cerebrospinal fluid is associated with multiple sclerosis progression. Mol Neurobiol 47(3):946–956. https://doi.org/10.1007/s12035-012-8387-1
23. Savonius O, Pelkonen T, Roine I, Viljakainen H, Andersson S, Fernandez J, Peltola H, Helve O (2018) Vitamin D was not associated with survival or cerebrospinal fluid cathelicidin levels in children with bacterial meningitis. Acta Paediatr 107(12):2131–2136. https://doi.org/10.1111/apa.14393
24. Zhang L, Ma L, Zhou X, Meng J, Wen J, Huang R, Gao T, Xu L, Zhu L (2018) Diagnostic value of Procalcitonin for bacterial meningitis in children: a comparison analysis between serum and cerebrospinal fluid Procalcitonin levels. Clin Pediatr 9922818809477:159–165. https://doi.org/10.1177/0009922818809477
25. Perga S, Giuliano Albo A, Lis K, Minari N, Falvo S, Marnetto F, Caldano M, Reviglione R, Berchialla P, Capobianco MA, Malentacchi M, Corpillo D, Bertolotto A (2015) Vitamin D binding protein isoforms and apolipoprotein E in cerebrospinal fluid as prognostic biomarkers of multiple sclerosis. PLoS One 10(6):e0129291. https://doi.org/10.1371/journal.pone.0129291
26. Pardridge WM, Sakiyama R, Coty WA (1985) Restricted transport of vitamin D and a derivatives through the rat blood-brain barrier. J Neurochem 44(4):1138–1141
27. Jereb M, Muzlovic I, Hojker S, Strle F (2001) Predictive value of serum and cerebrospinal fluid procalcitonin levels for the diagnosis of bacterial meningitis. Infection 29(4):209–212
28. Bennett J, Basivireddy J, Kollar A, Biron KE, Reickmann P, Jefferies WA, McQuaid S (2010) Blood-brain barrier disruption and enhanced vascular permeability in the multiple sclerosis model EAE. J Neuroimmunol 229(1–2):180–191. https://doi.org/10.1016/j.jneuroim.2010.08.011
29. Brown J, Bianco JI, McGrath JJ, Eyles DW (2003) 1,25-dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. Neurosci Lett 343(2):139–143
30. Bhan I, Powe CE, Berg AH, Ankers E, Wenger JB, Karumanchi SA, Thadhani RI (2012) Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients. Kidney Int 82(1):84–89. https://doi.org/10.1038/ki.2012.19
31. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG (1986) Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab 63(4):954–959. https://doi.org/10.1210/jcem-63-4-954
32. Bikle DD, Siiteri PK, Ryzen E, Haddad JG (1985) Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. J Clin Endocrinol Metab 61(5):969–975. https://doi.org/10.1210/jcem-61-5-969
33. Christensen EI, Birn H (2002) Megalin and cubilin: multifunctional endocytic receptors. Nat Rev Mol Cell Biol 3(4):256–266. https://doi.org/10.1038/nrm778
34. Nielson CM, Jones KS, Chun RF, Jacobs JM, Wang Y, Hewison M, Adams JS, Swanson CM, Lee CG, Vanderschueren D, Pauwels S, Prentice A, Smith RD, Shi T, Gao Y, Schepmoes AA, Zmuda JM, Lapidus J, Cauley JA, Bouillon R, Schoenmakers I, Orwoll ES (2016) Free 25-Hydroxyvitamin D: impact of vitamin D binding protein assays on racial-genotypic associations. J Clin Endocrinol Metab 101(5):2226–2234. https://doi.org/10.1210/jc.2016-1104
35. Jung JY, Choi DP, Won S, Lee Y, Shin JH, Kim YS, Kim SK, Oh YM, Suh I, Lee SD (2014) Relationship of vitamin D binding protein polymorphisms and lung function in Korean chronic obstructive pulmonary disease. Yonsei Med J 55(5):1318–1325. https://doi.org/10.3349/ymj.2014.55.5.1318
36. Mubashir M, Anwar S, Tareen AK, Mehboobali N, Iqbal K, Iqbal MP (2017) Association of vitamin D deficiency and VDBP gene polymorphism with the risk of AMI in a Pakistani population. Pak J

Med Sci 33(6):1349–1354. https://doi.org/10.12669/pjms.336.13379

37. Haldar D, Agrawal N, Patel S, Kambale PR, Arora K, Sharma A, Tripathi M, Batra A, Kabi BC (2018) Association of VDBP and CYP2R1 gene polymorphisms with vitamin D status in women with polycystic ovarian syndrome: a north Indian study. Eur J Nutr 57(2):703–711. https://doi.org/10.1007/s00394-016-1357-z

38. Knudsen CS, Nexo E, Hojskov CS, Heickendorff L (2012) Analytical validation of the Roche 25-OH vitamin D Total assay. Clin Chem Lab Med 50(11):1965–1968. https://doi.org/10.1515/cclm-2011-0964