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Cerebrospinal fluid analysis in 13 clinically healthy Beagle dogs; hematological, biochemical and electrophoretic findings

IL-Hwan Kim[1,4], Dong-In Jung[1], Jong-Hyun Yoo[2], Byeong-Teck Kang[1], Chul Park[3], Hee-Myung Park[1,*]

[1]*College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea*
[2]*BK21 Program of Integrative Network Systems for Veterinarians in Basic Science, Industrial Animals and Preventive Medicines, Konkuk University, Seoul 143-701, Korea*
[3]*School of Veterinary Medicine, University of California, Davis, Davis, California 95616, USA*
[4]*SamJeon Animal Hospital, Seoul 138-180, Korea*
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Abstract : The purpose of this study is to define the normal findings of cerebrospinal fluid (CSF) of the clinically healthy Beagle dogs and to provide basic information in diagnosis of neurologic disorders. CSF obtained from 13 clinically healthy dogs was examined for total and differential cell counts, total protein concentration, glucose and lactate dehydrogenase (LDH) concentration, specific gravity, turbidity, and protein electrophoresis. On gross examination, CSF samples evaluated were clear and colorless. Few red blood cells and nucleated cells were present. The mean concentration of glucose and LDH examined were 65.8 mg/dl and 2.7 mg/dl, respectively. The cellular components of CSF samples based on differential counts were monocytes (41.9%), activated macrophages (35.8%), lymphocytes (20.0%), neutrophils (1.6%), and eosinophils (0.7%). The fractions of electrophoretic protein in CSF were albumin (52.7%), alpha-globulin (16.5%), beta-globulin (24.8%), and gamma-globulin (3.0%). Results of albumin quota were ranged from 0.15 to 0.38. In conclusion, this study provided normal composition of CSF in Beagle dogs.

Keywords : beagle dog, cerebrospinal fluid (CSF), cerebellomedullary cistern

Introduction

In dogs, cerebrospinal fluid (CSF) analysis provides rapid and, in some situations, instant information to the veterinary clinician dealing with neurological disorders [15]. The data from the CSF analysis may be useful to identify the etiology of neurological disorders [3]. CSF evaluation alone is not often diagnostic, but it may be beneficial in differentiate diagnosis, if it is combined with other tests [3, 10].

When correctly performed under general anesthesia, cerebellomedullary cistern CSF collection in dogs is safe procedure and there are few harmful sequelae. The cisternal site is preferred for intracranial disease and the lumbar site is preferred for thoracic and lumbar spinal cord disease [14].

The purpose of this study is to define the normal findings of CSF of the clinically healthy Beagle dogs and to provide basic information in diagnosis of neurologic disorders.

Materials and Methods

Animals

Thirteen intact Beagle dogs (5 males, 8 females, 1-2 years, and 8.6-13.7 kg) were used in this study. All dogs were fed with the same diet (Jeroni; CJ, Korea). The food was withheld for 12 h prior to study. No abnormalities including the complete blood count (CBC), serum biochemistry and survey radiography were found in the dog. Fecal floatation for endoparasiticism showed no remarkable findings. All dogs were completely vaccinated prior to study (Canigen DH(A2)PPIL; Virbac, France).

*Corresponding author: Hee-Myung Park
College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea
[Tel: +82-2-420-4140, Fax: +82-2-450-3037, E-mail: parkhee@konkuk.ac.kr]

CSF collection procedure

Each dog was premedicated with 0.05 mg of atropine/kg of body weight subcutaneously (Jeil pharm, Korea). Anesthesia was maintained with ketamine (6 mg/kg; IV; Yuhan corporation, Korea) and xylazine (1.1 mg/kg; IV; Bayer Korea, Korea). CSF was collected by cerebellomedullary cisternal (CMC) puncture method with 22 gauge spinal needle.

Following the CSF tapping, the dogs were maintained on oxygen (100 ml/kg/min) until they required extubation.

Analysis of CSF

Color and turbidity of CSF were grossly examined and recorded. Nucleated cell counts were performed using standard hemocytometer technique [2]. All nucleated cells were counted in the 10 large squares (four corner squares and one center square on each side) for a total nucleated cell count per microliter.

Glucose and lactate dehydrogenase (LDH) concentration were determined using automatic serum chemical analyzer (Dry-chem clinical chemistry analyzer 3500i; FUJI, Japan) according to the manufacturer's instructions. In addition, specific gravity (SG) was measured by using refractormeter.

Cytological evaluation

As CSF has a low cellular content, a cytocentrifuge (Cytospin4; Thermo Shandon, UK) was employed instead of using a direct smear of CSF. The CSF sample was spun directly onto a glass slide at 250 g for 5 min. The slides were immediately dried, stained by the Diff-Quik stain (Sysmex corp., Japan) and examined under light microscopy. Briefly, differential cell counts were performed by classifying a minimum of 100 cells on each slide. Monocytes, activated macrophages, neutrophils, lymphocytes, eosinophils, and epithelial cells were classified according to the method described previously [1].

Protein determination and Protein electrophoresis

Samples were stored at −80°C until used. All samples were analyzed within 7 days. Total CSF protein determinations was performed along with bovine gamma-globulin as a standard and measured with a lowry protein assay [12] and a urinary reagent dipstick according to a previous method [9].

CSF was concentrated 100 times before protein electrophoresis. Electrophoresis was carried out for 15 min at 180 volts using cellulose acetate membranes. After electrophoresis, cellulose acetate membranes were stained with Ponceau S and washed with 5% acetic acid. Total protein was fractionated into albumin, α-, β-, γ-globulin then was quantified by scanning densitometer (Neodin Vetlab, Korea) and measured each concentrations. Serum albumin concentrations for albumin quota (AQ) in this study were measured with the same technique used in CSF.

Table 1. Color, turbidity, total protein, lactate dehydrogenase, and glucose concentration of CSF in 13 Beagle dogs were summarized

Dog No.	Color	Turbidity	TP* (UD†)	TP (L‡) (mg/dl)	LDH$ (mg/dl)	Glucose (mg/dl)
1	Colorless	Clear	1+	14	5	68
2	Colorless	Clear	1+	18	3	57
3	Colorless	Clear	1+	14	5	59
4	Colorless	Clear	1+	14	1	63
5	Colorless	Clear	1+	13	4	74
6	Colorless	Clear	1+	14	1	53
7	Colorless	Clear	1+	13	1	61
8	Colorless	Clear	Trace	8	1	104
9	Colorless	Clear	Trace	11	1	64
10	Colorless	Clear	Trace	12	1	60
11	Colorless	Clear	Trace	11	1	60
12	Colorless	Clear	1+	16	6	67
13	Colorless	Clear	1+	22	5	65
Mean ± SD				13.8 ± (3.4)	2.7 ± 2.0	65.8 ± 12.6

*TP: Total protein, †UD: Urine dipsticks, ‡L: Lowry assay, $LDH: Lactate dehydrogenase.

Table 2. Total and differential cell counts in CSF obtained from 13 Beagle dogs

Dog No.	TNC* (cells/μl)	MON† (%)	MAC‡ (%)	LYM§ (%)	NEU‖ (%)	EOS¶ (%)
1	3	16	57	19	8	0
2	1	51	40	8	0	1
3	2	61	24	12	0	3
4	1	18	38	38	6	0
5	4	49	39	10	2	0
6	3	49	29	22	0	0
7	3	47	36	17	0	0
8	2	35	48	14	2	1
9	1	51	5	44	0	0
10	2	34	49	14	2	1
11	3	75	22	2	1	0
12	3	40	30	28	0	2
13	2	18	49	32	0	1
Mean ± SD	2.3 ± 0.9	41.9 ± 17.5	35.8 ± 13.9	20 ± 12.3	1.6 ± 2.5	0.7 ± 0.9

*TNC: Total nucleated cells, †MON: Monocytes, ‡MAC: Activated macrophages, §LYM: Lymphocytes, ‖NEU: Neutrophils, ¶EOS: Eosinophils.

Results

Grossly, all CSF samples obtained through cerebellomedulary cistern were colorless and clear in this study (Table 1). Few red blood cells (RBCs) in CSF were present in some samples and total nucleated cells were ranged from 1 to 4 cells/μl (Table 2).

Glucose concentration of CSF was ranged from 53 to 104 mg/dl (65.8 ± 12.6 mg/dl). In addition, LDH concentration were all below 10 mg (1-6 mg/dl, mean; 2.7 ± 2.0 mg/dl).

SG of all CSF samples used was less than 1.005. For clinical use based on a previous report [9], total protein concentration was ranged from trace to 1+ (approximately 30 mg/dl) in urine dipstick test.

Mean differential cell count in CSF from the 13 Beagle dogs is summarized in Table 2. The majorities of cell presented were monocytes, activated macrophages and lymphocytes, while neutrophils, epithelial cells, and eosinophils were rarely observed.

There were no significant differences in mean total nucleated cell counts among the dogs evaluated. Similarly, variation in the number of eosinophils and neutrophils from all dogs studied was minimal.

However, the number of monocytes, activated macrophages, and lymphocytes was varied in dogs evaluated (Table 2).

Predominant types of cells were monocytes and

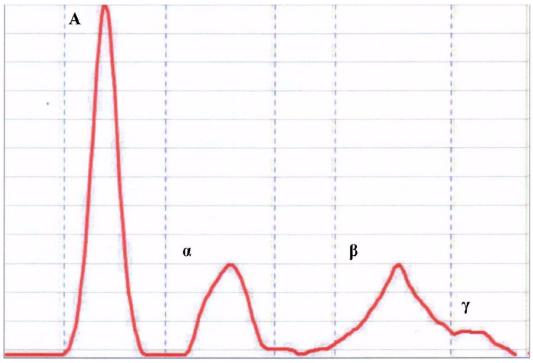

Fig. 1. Densitometric tracing of normal CSF after protein electrophoresis (Case No. 11). A = albumin (50.6%), α = α-globulin (19.2%), β = β-globulin (24.5%), γ = γ-glbulin (4.5%).

activated macrophages. Morphologically, well-differentiated monocytes were relatively large (10 to 15 μm), round to oval discrete cells with eccentric round to oval nuclei (Fig. 2A). Nuclear chromatin was usually condensed than that seen in other macrophages and single nucleoli were more common. Numerous cytoplasmic vacuoles of activated macrophages were observed frequently (Fig. 2B). Lymphocytes, neutrophils, and eosinophils were well preserved and were morphologically similar to those in peripheral blood (Figs. 2C and 2D). On the average, lymphocytes accounted for approximately 20% of the total nucleated cells in the CSF obtained from normal dogs, and

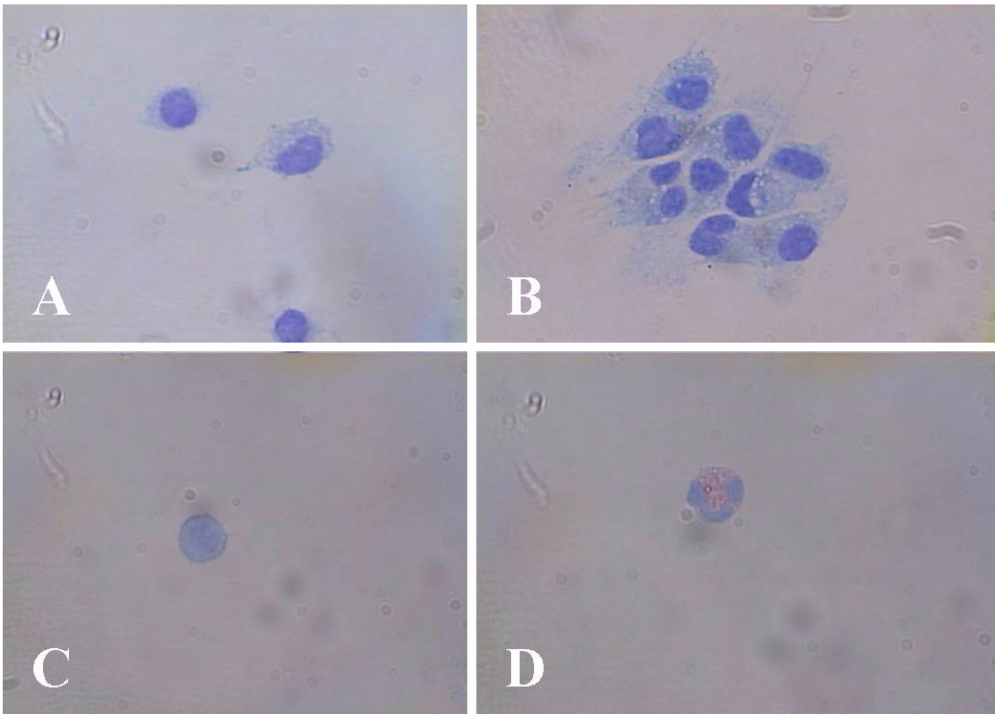

Fig. 2. Cerebrospinal fluid tapping from clinically normal Beagle dogs. A: Monocytes predominate and these cells show condensed nucleus and mildly vacuolated cytoplasm (Diff-Quik stain ×1,000). B: Activated macrophages predominate and these cells show pale foamy cytoplasm or highly vacuolated (Diff-Quik stain ×1,000). C: A lymphocyte cell is present (Diff-Quik stain ×1,000). D: An eosinophil is present (Diff-Quik stain ×1,000).

Table 3. Results of CSF protein electrophoresis in 13 Beagle dogs

Items tested (fraction)	This study (n = 13) Mean ± SD (%)	Previous study (n = 10)* Mean ± SD (%)
Albumin	52.7 ± 8.9	37 ± 4.29
Alpha-globulin	16.5 ± 5.4	28 ± 5.27
Beta-globulin	24.8 ± 7.8	25 ± 5.31
Gamma-globulin	3.0 ± 1.5	7.75 ± 1.84

*Cited from Sorjonen DC, 1987 [16].

neutrophils and eosinophils composed of 3% or less of the differential cell count. Ependymal lining cells were rarely observed in total number of nucleated cells recovered.

Comparison of CSF electrophoresis results of our study to other study [16] was summarized in Table 3. The albumin fractions in both studies were higher than globulins. The CSF albumin was ranged between 35.8 and 65.5% (4.2 to 13.3 mg/dl) in this study. The globulin percentage of the total protein in the CSF of

Table 4. Results of serum albumin and albumin quota (AQ) ratio.

Dog No.	Serum albumin (mg/dl)	CSF* albumin (mg/dl)	AQ†
1	3100	6.7	0.22
2	3100	10.9	0.35
3	3200	11.1	0.34
4	2700	7.4	0.27
5	3100	9.2	0.29
6	3100	7.5	0.24
7	3000	6.9	0.23
8	2800	4.2	0.15
9	2900	6.5	0.22
10	2800	5.5	0.19
11	2900	5.6	0.19
12	2800	6.1	0.21
13	3500	13.3	0.38
Mean ± SD	2990 ± 200	7.7 ± 2.6	0.25 ± 0.07

*CSF: cerebrospinal fluid, †AQ = CSF albumin (mg/dl) × 100 / Serum albumin (mg/dl).

healthy dogs in this study was 9 to 23.9% (alpha fraction), 19.8% to 36.7% (beta fraction), 1.5% to 6.4% (gamma fraction), and in previous study was 24 to 31% (alpha fraction), 19 to 30% (beta fraction), 6 to 9% (gamma fraction) (Fig. 1). Results of AQ were ranged from 0.15 to 0.38 (Table 4).

Discussion

According to a previous report, few red blood cells (RBC) are present in CSF of healthy dogs, compared to dogs having pathological hemorrhage into the subarachnoid space [17]. In general, the increased number of RBCs in CSF sample could be resulted from needle puncture of blood vessels on the dura, and particularly the leptomeninges, during the sampling procedure. Pathological hemorrhage within the CSF is most confidently diagnosed by the presence of phagocytosed red blood cells within macrophages [18]. This result indicates that CSF tapping of the present study was correctly conducted, since there are rare red blood cells in this study.

Total protein concentration in this study were in accordance with those described previously [2-5, 10, 13], which reported values lower than 30 mg/dl in normal dogs.

Total nucleated CSF cells in healthy dogs were fewer than 5 cells/μl as reported in literatures [3, 4, 6, 13] indicating that there was no inflammatory lesion in central nervous system at that time of study.

Generally glucose concentration of CSF is normally about 80% of blood level [3, 5]. Based on results of the present study, glucose concentration of CSF was found to be lower than that of serum. This is similar with results of the previous studies [1, 3-5, 13]. As seen in other studies [6, 8], LDH was approximately 10 times lower than in serum [6]. LDH in CSF can be increased in lymphosarcoma affecting nervous system parenchyma [5, 7]. Thus LDH measurement can be used in diagnosis of CNS lymphoma.

Results of CSF electrophoresis in this study showed mild difference with previous report [16]. The author suggests that there will be some variation with each laboratory and technique used. For this reason, these differences between our study and previous study were not remarkable.

Because albumin is exclusively of serum origin, increased CSF albumin may be used to assess the integrity of the blood brain barrier. The amount of albumin in the CSF may vary with the serum albumin, and the calculation of a ratio between CSF and serum albumin, called an AQ, has been suggested as a more meaningful measure than CSF albumin alone [3]. Normal AQ for 13 dogs in this study was ranged from 0.15 to 0.38 (Mean ± SD; 0.25 ± 0.07) (Table 4). These results are similar with results of previous studies [3, 11, 16].

Knowledge of the AQ can be useful to interpret accurately changes in CSF alpha, beta, and gamma globulin fractions. Changes in CSF globulin percentages may be associated with transudation from serum, local production, or both. Alpha globulin present in normal CSF is derived only from serum, but beta and gamma globulins may be either derived from serum or locally produced [3, 16].

Previous studies [3, 4, 6, 10, 11, 14] showed that monocytes were predominant in normal CSF from healthy dogs and cats. One report [14] indicated monocytes compose 69 to 100% of the nucleated cells, lymphocytes 0 to 27%, neutrophils 0 to 9%, macrophages 0 to 3%, and eosinophils 0 to less than 1% of nucleated cells in healthy cats. In this study, monocytes were well differentiated and predominated (Table 2).

In this study, slides were prepared for staining by cytocentrifugation due to low cellularity of CSF. The cytologic findings in CSF of healthy dogs in this study were similar to that in CSF from other studies [1-4, 11, 13, 14].

According to the previous studies [1-4, 11, 13, 14] performed in dogs, there was some variation of cell populations depending on the collection site of the CSF. In particular, spinal cord disease cannot be diagnosed by cerebellomedullary cisternal puncture. In a retrospective study of seven dogs with thoracolumbar spinal cord neoplasia [16], mean CSF protein concentration were 33 mg/dl and 145 mg/dl in CMC and lumbar CSF samples, respectively. White blood cells (WBC) counts were higher in the lumbar CSF (5 to 11 cells/μl) than CMC CSF (0 to 1 cells/μl). In the same study, 73 CMC and 17 lumbar unpaired CSF samples from cases of thoracolumbar spinal cord compression from intervertebral disk extrusion and vertebral fractures and dislocations were compared. CSF in the lumbar region had elevated protein and increased WBC more frequently than CMC CSF. Based on the previous studies [3, 18], it might be

suggested that CSF from the lumbar subarachnoid space may reflect greater change and have greater diagnostic significance than CMC CSF in cases of focal thoracolumbar spinal cord disease. Further studies of CSF analysis on paired CSF samples from each site from dogs with neurologic disease are needed to determine if lumbar CSF samples consistently give different or more valuable information than CMC CSF.

Many studies about normal findings of CSF in dogs were previously reported in other countries [1-11, 13, 14, 16, 17]. However, because of predominant neurologic diseases, favorite species and general environmental differences depending on the places that the individuals live, normal CSF reference range of their own country should be necessary.

In conclusion, this study provided normal composition of CSF in Beagle dogs. In addition, this information would be useful to diagnose the neurological disorders of dogs.

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