

CEREBROSPINAL FLUID CONSTITUENTS IN HEALTHY FEMALE DROMEDARY CAMELS

F. A. AL-SOBAYIL

Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine,
Al-Qassim University, Buraydah, P. O. Pox 1482, Al-Qassim, Saudi Arabia

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SUMMARY

The appropriate location for collecting cerebrospinal fluid (CSF) from the dromedary camel was described and the reference range of cytological and biochemical constituents of CSF were determined in 25 clinically normal adult camels and compared with the corresponding serum. Camel CSF is colorless with similar viscosity to water. The maximum normal of RBCs and WBCs were 141 and 9 cells/ul, respectively. Significant differences were found in the biochemical values between the CSF and serum. These included total protein (TP), albumin, globulin, glucose, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT). In addition, there was significant difference between the CSF and serum in the levels of calcium.

Keywords: Cerebrospinal fluid, Serum, Cytological, Biochemical, Camels

INTRODUCTION

Determination of cerebrospinal fluid (CSF) constituents is valuable for diagnosis of neurologic diseases in humans and animals. In humans, normal values for CSF have been compared with the abnormal values obtained from individuals with neurologic diseases (Kjeldsberg and Knight, 1993; Ravel, 1995). Normal values for CSF have also been reported in horses (Mayhew et al, 1977; Mayhew et al, 1978, Andrews et al, 1990; Furr and Bender, 1994), cattle (Scott et al, 1990; Welles et al. 1992) buffaloes (Lal and Verma, 1979), llamas (Welles et al. 1994), goats (Smith, 1982; Swaroop et al, 1988), sheep (Beal and Bligh, 1977; Seoane, 1981), dogs (Wright, 1978; Jamison et al, 1988), and cats (Rand et al, 1990).

The composition of CSF obtained from animals with various neurologic and non-neurologic diseases have also been determined, and compared with the reference normal values (Garguez et al, 1971; Bijlveld and Binkhorstui 1973; Vandevelde et al, 1977; Bailey and Higgins, 1986; Sorjonen, 1987; Tvedten, 1987; Scott et al, 1990; Sarode et al, 1992; Patra et al, 1993).

The objective of the present study was to describe the appropriate location for collecting CSF from the dromedary camel and to provide reference range of cytological and biochemical values of the CSF in clinically healthy adult female dromedary camels and compare these values with the corresponding serum levels.

MATERIALS AND METHODS

Camels

Twenty five adult clinically healthy female camels aged between 6-9 years were used in this study. Body temperature, respiratory rate, heart rate, cranial nerve function, gait, and locomotion were assessed prior to CSF collection.

CSF collection

CSF samples were collected under general anesthesia from the atlanto-occipital space using 18 or 16-gauge 3.5 inches disposable spinal needles (Becton, Dickinson & Co, Franklin Lakes, NJ, USA). Xylazine (0.1 mg/kg IV) was first injected for sedation and followed by general anesthesia

with ketamine (2 mg/kg IV). Each camel was placed on the lateral recumbency and the hair was clipped over the poll and neck from between the ears to 4 inches caudally and approximately 2 inches on either side of the mane, and the area was aseptically prepared for the aspiration of CSF. The neck was flexed toward the sternum and the site of puncture was determined by locating a region just caudal to the point of intersection of an imaginary line drawn at right angles between the caudal border of the occipital bone and dorsal midline of the neck (Figure 1). Approximately 5-10 ml of CSF were withdrawn and immediately placed in a clean sterilized tube.

Blood samples were collected from the jugular vein immediately after collecting the CSF sample and transferred to plain vacutainer tubes. They were centrifuged at 3000 rpm for 10 minutes to obtain serum.

Laboratory analysis

CSF samples were immediately transferred to the laboratory. Red and white blood cells counts were determined using hemocytometer. The remainder of each CSF sample was centrifuged at 3000 rpm for 10 minutes and the supernatant was used for biochemical analysis. Smears were taken from the sediment on slides, and after air drying, stained with Wright Giemsa stain. The stained slides were used to determine the differential WBCs. The concentrations of total protein, albumin, globulin, glucose, calcium, and enzymes including lactate

dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) in both CSF and serum samples were determined by an Automated chemical analyzer (BM/HITACHI 902). The concentrations of sodium and potassium were measured by using the flame photometry (Flame photometer, Ontario, Canada).

Statistical analysis

Mean, SD, median, maximum and minimum values, and 95% confidence intervals of the mean were calculated for all measured CSF and serum

variables. Analysis of variance (ANOVA) was used to determine the differences in the dependent variables between CSF and serum samples, and the significance level was set at $P < 0.05$.

RESULTS

Normal camel CSF is clear, colorless, with approximately the same viscosity of water. The summary of CSF cytology results is shown in Table I, whereas the summary of biochemical constituents of both CSF and serum is shown in Table II.

Table (I): Cytology of normal CSF obtained from healthy camels

Variable	Mean \pm SD	Min	Max	95% CI
RBCs cells/ul	64.61 \pm 33.97	20	141	48.92 - 80.30
WBCs cells/ul	3.72 \pm 2.96	0	9	92.35 - 5.09
Neutro %	12.64 \pm 17.15	0	44.44	4.71 - 20.56
Neutro number	0.83 \pm 1.29	0	4	0.24 - 1.43
Lymph %	58.57 \pm 33.27	0	100	43.20 - 73.94
Lymph number	2.28 \pm 1.49	0	4	1.59 - 2.97
Mono %	11.24 \pm 14.25	0	50	6.58 - 17.82
Mono number	0.56 \pm 0.61	0	2	0.27 - 0.84
Eosino %	0.94 \pm 4.01	0	17	0 - 2.80
Eosino number	0.06 \pm 0.24	0	1	0 - 0.16

Table 1: The levels of biochemical variables in serum and CSF of camels.

Variable	Mean \pm SD	Median	Min	Max	95% CI
GTP: serum (g/dl) CSF (mg/dl)	6.53 \pm 0.40* 54.70 \pm 13.10	6.47 55.65	5.92 30.5	7.25 77.20	6.35, 6.72 48.65, 60.75
Albumin: serum (g/dl) CSF (mg/dl)	3.49 \pm 0.36* 22.53 \pm 6.46	3.37 23.15	3.15 11.30	4.31 33.90	3.31, 3.65 19.55, 25.52
Albumin Quota	0.64 \pm 0.15	0.69	0.34	0.82	0.57-0.71
Globulin: serum (g/dl) CSF (mg/dl)	2.87 \pm 0.22* 15.77 \pm 2.97	2.90 16.30	2.42 10.50	3.25 20.6	2.76, 2.97 14.39, 17.14
Glucose: serum (mg/dl) CSF (mg/dl)	93.94 \pm 13.09* 61.67 \pm 17.12	9 55	72 40	116 90	90.87, 99.99 53.76, 69.58
ALP: serum (IU) CSF (IU)	54.28 \pm 8.03* 9.11 \pm 8.44	67 6.5	48 1	76 30	60.57, 67.99 5.22, 13.01
LDH: serum (IU) CSF (IU)	355.38 \pm 90.92* 15.77 \pm 4.44	360.5 16.80	215 8.97	562 23.04	13.73, 17.83
CK: serum (IU) CSF (IU)	72.89 \pm 10.57* 11.59 \pm 5.41	74 12.55	54 1.63	89 21.02	68.00, 77.77 9.09-14.09
GPT: serum (IU) CSF (IU)	10.88 \pm 2.85* 5.11 \pm 4.86	10 4	7 0	16 17	9.57, 12.21 2.87, 7.36
GOT: serum (IU) CSF (IU)	66.56 \pm 11.38* 6.06 \pm 3.90	63 5	52 1	86 16	61.30, 71.81 4.25 - 7.86
Calcium: serum (mg/dl) CSF (mg/dl)	8.69 \pm 0.50* 4.26 \pm 0.61	8.52 4.20	8.13 3.30	0.74 5.30	8.46, 8.93 3.97 - 4.54
Sodium: serum (mmol/l) CSF (mmol/l)	151.33 \pm 3.36 153.17 \pm 1.47	150 153	148 151	159 156	149.78, 152.89 152.49, 153.85
Potassium: serum (mmol/l)	4.52 \pm 0.62 3.32 \pm 0.13	4.51 3.3	3.5 3.2	5.3 3.6	4.23, 4.80 3.26 - 3.38

* Indicates statistically significant difference (P < 0.05).

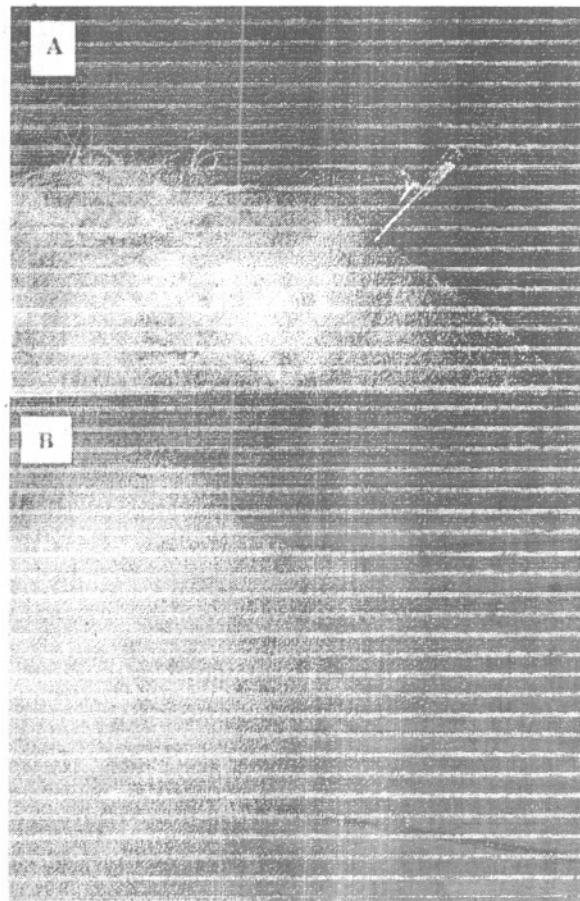


Fig. 1. A. The site of collecting CSF from camels. B. A radiograph of the skull showing the needle in the appropriate region.

DISCUSSION

CSF collection from dromedary camels was not difficult, and it can be routinely performed in the anesthetized camels suspected of neurologic diseases. In all camels, recovery was smooth without complications. In the present study, the hemocytometer was used to determine CSF cell counts. It has been reported that the determination of CSF cell counts (RBCs & WBCs) and differential cell count should be performed manually and an electronic cell counters should not be used because their precision is poor in determining the

normal ranges (Kjeldsberg and Knight, 1993). It is assumed that the RBCs content of CSF is zero unless hemorrhage occurs. However, few numbers of RBCs were observed in all samples. This probably occurred as a result to traumatic tapping when collecting the CSF. Traumatic puncture and pathologic hemorrhage can be differentiated by centrifuging a CSF sample. If the red blood cells disappear in the pellet leaving a clear colorless fluid, this indicates that the hemorrhage is due to needle puncture. Meanwhile, if there is a clear sample of CSF with a dark yellow or pale amber color (xanthochromic), this likely represents

previous hemorrhage which might be an indication of prolonged and low-grade hemorrhage or massive hemorrhage at some time prior to aspiration of the CSF. In the present study, the hemorrhage occurred in the CSF samples was caused by needle puncture. A more recent sensitive and specific test has been described to differentiate the pathologic hemorrhage from traumatic puncture (Lang et al., 1990). In this test, a commercial latex agglutination immunoassay is used to measure the levels of a cross-linked fibrin derivative D-dimer which is usually present in CSF samples from patients with pathologic hemorrhage and not in CSF specimens associated with traumatic tap (Lang et al, 1990).

WBC counts of camel CSF were found to be as high as 9 cells/ul (3.72 (2.96 cells/ul). This value is similar to what was reported in cattle (Welles et al, 1992) and larger than the reported values of adult humans (Ravel, 1995) and other species (Welles et al, 1994; Mayhew et al, 1978; Mayhew et al, 1977; Jamison et al, 1988, Rand et al, 1990). On the other hand, this value is lower than the reference values of human newborns (Ravel, 1995). Differential leukocyte percentages in the CSF collected from camels showed that the lymphocytes (58.57 (33.27) were the predominant followed by the neutrophils (12.64 (17.15) and the monocytes (11.24 (14.25). Such values were similar to those reported for llamas (Walles et al, 1994). A significant positive correlation ($P < 0.05$) was found between the amount of blood

contamination and the number of neutrophils in camel CSF samples. This was similar to what was reported in llamas (Walles et al., 1994). However, this study showed that the percentage of neutrophils in camels CSF was lower than what Walles et al. (1994) reported in llamas. This may be due to species differs or levels of blood contamination. An eosinophil was found in only one sample of CSF. Blood contamination was thought to be the source of this eosinophil. Basophils were not found in the CSF. There is no general agreement as to what constitutes the normal upper limit for polymorphonuclear leukocytes (PMNs) in CSF. In humans, some workers consider 7% to 8% PMNs as normal limits if the total WBCs is normal (Krieg and Kjeldsberg, 1991). Others consider more than 2% PMNs as abnormal unless the peripheral blood PMN count is abnormally high (Hayward and Oye, 1988).

CSF protein is derived mostly from plasma. It has been reported that prealbumin, transferrin, and small quantities of nerve tissue-specific proteins are the major qualitative differences between CSF and plasma proteins of humans (Kjeldsberg and Knight, 1993). The concentration of total protein in camel CSF was found to be higher than those of cattle (Welles et al, 1992), goats (Altman and Dittmer, 1974), pigs (Altman and Dittmer, 1974), llamas (Welles et al, 1994), and dogs (Wright, 1978; Sorjonen et al, 1981), and lower than human values (Ravel, 1995) and horses (Furr et al, 1994; Andrews et al, 1990; Mayhew

et al, 1978). Other reports showed that adult humans and horses have less CSF TP than camels (Ravel, 1995; Mayhew et al, 1977). This is maybe due to various factors such as the different measurement method used, the different age and gender of camels used in both studies. The effects of measuring method used, age, and gender are considered important variables that can affect normal values of CSF TP (Tibbling et al, 1977; Breebaart et al, 1978; Kjeldsberg and Knight, 1993). In addition, the amount of CSF contaminated with blood as a result of traumatic tapping is thought to be a common cause of increased CSF TP (Kjeldsberg and Knight, 1993). It has been reported that the rise in the levels of human CSF TP was about 400 mg/dL when 1 ml of CSF is contaminated with 0.1 ml of blood (Krieg and Kjeldsberg, 1991). However in our study, no correlation was observed between blood contamination and total protein concentration in CSF. On the other side, there was a positive correlation in the TP concentration between the CSF and serum proteins. This study showed that the CSF normally contains less than 1% of the amount of protein present in serum. Similar result has been reported in humans (Kjeldsberg and Knight, 1993). In this study, albumin concentration represented approximately 23 % of the total protein in CSF. This is slightly higher than what was reported in llamas (Welles, 1994) and pigs (Altman, 1974), cattle (Welles, 1992), and significantly higher than what was reported in dogs (Sorjonen et al, 1981) and cats (Hochwald et al, 1969). The con-

centration of albumin in camel CSF was found to be less than that of horses (Andrews, 1990). There was a positive correlation between the concentration of TP and albumin in camels CSF. In this study, albumin quotient was higher than what was reported for llamas (Walles, 1994), cattle (Walles, 1992), and dogs (Sorjonen, 1987), and lower than that of horses (Andrews, 1990). Albumin quotient has been used to assess the function of blood-brain barrier. In general, the blood-brain barrier is altered when the levels of albumin quotient is > 2.35 (Duncan et al, 1994). The concentration of globulin in CSF of camels was close to that reported for dogs (Sorjonen et al, 1981), but less than that for llamas (Welles et al, 1994) and horses (Fankhauser, 1962). The level of globulin in CSF of camels was significantly lower than its concentration in serum. The concentrations of globulin in CSF represented approximately 0.55% of its concentration in serum.

The CSF glucose concentration is dependent on its level in the blood as it is derived entirely from plasma. In camels, the concentration of CSF glucose was close to what was reported in llamas (Welles, 1994), goats (Altman and Dittmer, 1974) and horses (Mayhew et al, 1977); higher than its value in humans (Ravel, 1995) and cattle (Welles et al, 1992); lower than its value in dogs (Wright, 1978) and cats (Fankhauser, 1962). The present study showed that there was a fluctuation between the CSF and serum glucose concentrations. The concentration of CSF glucose represented

approximately 66% of the blood glucose level. This is within the reported accepted level which is 60-80% (Duncan et al, 1994).

Various enzymes were found in camel CSF. In humans, it has been reported that the CSF enzymes have three possible sources: brain tissue or CNS tumors, blood, and cellular elements within the CSF (Kjeldsberg and Knight, 1993). The lack of reliable CSF enzymes reference values is a major problem negatively affects the usefulness of evaluating CSF enzymes in camels. Evaluation of CSF enzymes is a valuable indicator that can be used in diagnosis and prognosis of various neurologic diseases. The level of ALP in camel CSF was much higher than what was reported in horses (Mayhew et al, 1977), and it basically represented around 17% of the serum ALP. LDH has been the most frequent enzyme studied in CSF. The concentration of LDH in camel CSF was approximately similar to what was reported in cattle (Welles et al, 1992), llamas (Welles et al, 1994) and higher than what was found in cats (Rand et al, 1990), and dogs (Coles, 1980) and higher or lower than what was reported in horses (Rossdale et al, 1982; Mayhew, 1977). The percentage of LDH in CSF of camels represented less than 4.5% of its level in the serum. This is less than what was reported in humans (Kjeldsberg and Knight, 1993). GOT level in CSF of camels was approximately close to what was reported in other domestic animals (Mayhew et al, 1977; Andrews et al, 1990; Rossdale et al, 1982; Coles,

1980). About 10% of serum GOT was found in CSF of healthy camels. It has been reported that the CSF and serum GOT levels vary independently (Kjeldsberg and Knight, 1993). GPT level in CSF of camels was less than that of dogs (Coles, 1980). About 47% of serum GPT was found in camels CSF.

CK is mostly abundant in mammalian skeletal muscle, cardiac muscle and neural tissue (Dawson et al, 1967). It is reported that plasma CK does not cross the blood-brain barrier in normal situations and normal CK in CSF appears to be derived from the central nervous system (Sherwin et al, 1969). This study showed that the level of CK in CSF from camels was higher than what was reported in horses (Mayhew et al, 1977; Rossdale et al, 1982) and similar than that in cattle (Welles et al, 1992). Approximately 16% of serum CK was found in camels CSF. The present study showed that there were significant differences in constituents of TP, albumin, globulin, glucose, and all the enzymes between their levels in CSF and serum.

The sodium ion is the major electrolyte present in both blood and CSF. The concentration of sodium in CSF of camels was slightly higher than that of humans (Kjeldsberg and Knight, 1993), horses (Rossdale et al, 1982; Mayhew et al, 1977), and cattle (Welles et al, 1992). It was close to what was reported for llama (Welles et al, 1994), and dogs. CSF sodium ion of camels

was slightly higher than its concentration in the blood. This finding is basically found in all domestic animals (Duncan et al, 1994). The CSF potassium concentration of camels was similar to what was reported in humans and other domestic animals (Mayhew et al, 1977; Rossadle et al, 1982; Welles et al, 1992; Kjeldsberg and Knight, 1993; and Welles et al, 1994). About 73% of blood potassium is found in the CSF of camels. In camels, CSF calcium was similar to what was reported in horses (Mayhew et al, 1977); and less than what was found in sheep (Altman and Dittmer, 1974), dogs and cats (Fankhausor, 1962). A significant difference was found in the level of calcium in serum and CSF, and approximately 50% of serum calcium was found in CSF of camels. Similar result was reported in humans (Kjeldsberg and Knight, 1993).

CONCLUSION

The reference range values of cytological and biochemical constituents of CSF collected from healthy adult dromedary camels and compared with that of the serum levels were reported. The WBC counts of camel CSF were similar to that in cattle and the differential cytology was close to what was reported in llamas. The concentration of TP and albumin in CSF of camels were higher than that in ruminants and lower than that in horses. On the other hand, the concentration of globulin in CSF of camels was less than what was reported in llamas and horses. The concentrations

of CSF glucose and GOT in camels were close to what was reported in farm animals. The level of ALP in CSF of camels was higher than its levels in CSF from horses. The levels of LDH and CK in camel CSF were similar to what was reported in cattle. The concentrations of sodium and potassium in CSF from camels were close to what was reported in llamas. The levels of calcium in CSF of camels were similar to what was reported in humans. There were significant differences between the CSF and serum in the concentrations of TP, albumin, globulin, glucose, ALP, LDH, CK, GOT, GPT, and calcium.

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مكونات السائل المخى الشوكى فى إناث الإبل وحيدة السنام السليمة صحياً

فهد عبدالله السبيل

قسم الطب البيطرى ، كلية الزراعة والطب البيطرى ، جامعة القصيم ، بريده،

ص . ب . ١٤٨٢ ، القصيم، المملكة العربية السعودية

لقد تم وصف المكان المناسب لأخذ عينة السائل المخى الشوكى من الإبل كما وتم تحديد المحتويات الخلوية وبعض المكونات البيوكيميائية للسائل المخى السوكى لـ ٢٥ ناقة بالغة سليمة صحياً وتم مقارنتها بقيمتها الموجودة فى المصل. السائل المخى الشوكى للإبل لالون له وله نفس لزوجة الماء. أقصى القيم لكرات الدم الحمراء والبيضاء فى السائل المخى السوكى للإبل كانت ١٤١ خلية/ميكروليتر و٩ خلايا/ميكروليتر على التوالي. وجد هناك فروق معنوية فى المكونات البيوكيميائية بين السائل المخى الشوكى ومصل الدم وهذه المكونات شملت تركيز كل من البروتين الكلى، الألبومين، الجلوبيولين، الجلوكوز، الألكلين فوسفاتيز، لاكتيت ديهيدروجينيز، كرياتينين كايينيز، جلوتامك بيروفك ترانسامينيز، جلوتامك أوكسال أستيك ترانسامينيز. بالإضافة إلى ذلك فقد كان هناك فروق معنوية فى مستوى الكالسيوم بين السائل المخى الشوكى ومصل الدم.