犬糸状虫性血色素尿症に関する研究,実測および算出血清浸透圧ギャップ

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Clinical Studies on Canine Dirofilarial Hemoglobinuria: Measured and Calculated Serum Osmolalities and Osmolar Gap

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ABSTRACT. Serum osmolalities and osmolar gap were determined in 43 normal healthy beagles (control group) and 40 dogs with dirofilarial hemoglobinuria (hemoglobinuria group). In the control group, the measured and calculated serum osmolality levels were in the means of 296±5 (SD) mOsm/kg and 293±6 mOsm/kg respectively, showing an osmolar gap less than 10 mOsm/kg. In the hemoglobinuria group, the measured serum osmolality ranged from 272 to 370 mOsm/kg. A considerable number of dogs had normal serum osmolalities in spite of severe intravascular hemolysis, suggesting that the changes in serum osmolality would not be the direct cause of intravascular hemolysis. The measured serum osmolality (331±28 mOsm/kg) was significantly higher in 11 dogs which died after a surgical removal heartworms than in 29 dogs which recovered after the removal (302±17 mOsm/kg). The calculated serum osmolality level was 296±16 mOsm/kg in 24 recovered cases, and 304±22 mOsm/kg in 10 fatal cases. The osmolar gap stayed in the normal ranges of 5.4±5.9 mOsm/kg in recovered cases, but it attained a higher level of 22.7±8.9 mOsm/kg in fatal cases, suggesting poor prognosis in cases with large osmolar gaps. There were significant positive correlations between the measured serum osmolality and osmolar gap, serum sodium, potassium, BUN, GOT, GPT, creatinine, bilirubin and plasma hemoglobin values, as well as between the osmolar gap and serum potassium, BUN, GOT, GPT, creatinine and bilirubin values. The plasma hemoglobin concentration fell markedly without significant change in serum osmolality 20 hr after the heartworm removal.—KEY WORDS: dirofilarial hemoglobinuria, dog, osmolar gap, serum osmolality.

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Osmometry has been applied for evaluation of fluid and electrolyte disorders such as shock, dehydration, diabetes and certain drug intoxication [2, 3, 18, 23] in human medicine, but it has not been utilized very often in veterinary medicine [9, 11, 19, 20]. The serum osmolality can be measured directly with an osmometer (measured osmolality), but it can also be calculated from the concentrations of the principal osmotically active substances in serum (calculated osmolality). The osmolar gap, the arithmetic difference between the measured and calculated osmolalities, also provides useful information on the diagnosis and prognosis of the disease [7, 16, 21, 22].

Dogs with dirofilarial hemoglobinuria (caval syndrome) have severe circulatory disturbances, intravascular hemolysis and injuries of the liver, kidney and other organs [12, 14, 15, 17]. These disorders influence the serum osmolality, and in turn, the deviation in serum osmolality may also bring about intravascular hemolysis as in paroxysmal hemoglobinuria in calves [20]. There has been, however, no report on the serum osmolality in dogs with dirofilariasis. The present study describes the measured and calculated serum osmolalities and osmolar gap determined in normal dogs and dogs with dirofilarial hemoglobinuria. Clinical significance of these parameters is discussed.

MATERIALS AND METHODS

Ten female and 30 male dogs with proved dirofilarial hemoglobinuria were used (hemoglobinuria group). The age of the dogs ranged from 2.5 to 10 years. They displayed signs of so-called caval syndrome, that is, systolic cardiac murmur, jugular pulse, hemoglobinuria, hemoglobinemia, anemia, dyspnea and prostration. Ascites was observed in 10 cases. Heartworms were removed surgically through the jugular vein [8] in all dogs. Twenty-nine dogs recovered, but 11 died after the surgery. The pre- and post-operative data were compared in 7 fatal cases. The control group consisted of 43 healthy beagles including 20 females and 23 males. These animals, with ages of 11 months to 6 years old, bred in the C. S. K. Laboratory Animals Co., Ltd. (Minowa-cho, Nagano).

The peripheral blood was collected from the jugular vein before and 20 hr after the heartworm removal. The serum was separated immediately. Measured serum osmolality was determined with a vapor pressure osmometer (Model 5100-B, Wescor Inc., U.S.A.). Calculated osmolality was obtained by the following formula [3]:

Calculated osmolality (mOsm/kg) = sodium concentration (mEq/*l*) $\times$ 1.86 + BUN concentration (mg/d*l*)／2.8 + glucose concentration (mg/d*l*)／18 + 5. The osmolar gap was obtained by subtracting the calculated osmolality from the measured. Serum sodium and potassium concentrations were measured with a flame photometer (Model 205, Hitachi Ltd., Tokyo). The concentrations of BUN, glucose and creatinine were determined by the urease-indophenol method, the glucose oxidase assay, and the alkaline picric acid method, respectively (RaBA system, Chugai Pharmaceutical Co., Ltd., Tokyo). The plasma hemoglobin concentration was measured by the benzidine reaction. Other laboratory tests were done following the procedures described previously [12, 13]. For comparison of the data, Duncan's new multiple range test or paired *t*-test was used.

RESULTS

The laboratory test results in the control and hemoglobinuria groups are shown in Table 1. The hemoglobinuria group revealed severe anemia, an increase in WBC, decreases in albumin and A/G ratio and higher GOT, GPT, creatinine, bilirubin and plasma hemoglobin levels.

Table 2 represents the serum osmolalities and osmolar gap in the normal group and pre-operative hemoglobinuria group including recovered and fatal cases. The control group gave the measured and the calculated osmolality of 296±5 (mean±SD) mOsm/kg and 293±6 mOsm/kg respectively, and the osmolar gap was 1.5±4.9 mOsm/kg. In dogs died after the heartworm removal, the measured serum osmolality level at pre-operation was significantly higher than in the control group ($p<0.01$) and in those recovered after heartworm removal ($p<0.01$). However, calculated osmolality was not statistically different among the 3 groups. The osmolar gaps were also greater in fatal cases than in the controls ($p<0.01$) and cases of recovery ($p<0.01$). There were no significant differences in serum osmolalities and osmolar gap between the cases with and without ascites (data not shown).

Individual values of measured serum osmolality are plotted in Fig. 1. In the control group, the osmolality levels ranged from 289 to 308 mOsm/kg. The levels varied from 272 mOsm/kg at the lowest to 347 mOsm/kg at the highest in recovered cases, but from normal (298 mOsm/kg) to extremely higher (370 mOsm/kg) in fatal cases of the hemoglobinuria group. The individual values of calculated serum osmolality and osmolar gap in the control and the hemoglobinuria group are shown in Fig. 2. For the controls and the cases of recovery, the calculated osmolality values agreed with those measured, which were lower than those measured in fatal cases. The osmolar gap in the control group was 10 mOsm/kg or less. The majority of cases that recovered

Table 1. Hematological and serum biochemical test results in the control and hemoglobinuria groups

Parameter	Control		Hemoglobinuria		p<[b)]
	n	Mean±SD[a)]	n	Mean±SD	
RBC ($\times 10^4/\mu l$)	42	771±81 (635-928)[c)]	40	331±114 (127-515)	0.01
Ht (%)	42	52.0±9.0 (41.5-60.5)	40	22.6±6.9 (9.5-33.5)	0.01
Hb (g/d*l*)	42	17.3±1.8 (13.0-20.9)	40	7.1±2.4 (2.9-10.9)	0.01
WBC ($\times 10^2/\mu l$)	42	117±22 (83-161)	40	225±84 (51-410)	0.01
Total protein (g/d*l*)	43	6.6±0.4 (6.0-7.4)	40	7.3±0.9 (5.5-10.2)	0.01
Albumin (g/d*l*)	43	3.48±0.31 (3.14-3.83)	40	2.34±0.42 (1.2-3.1)	0.01
A/G ratio	43	1.12±0.19 (0.74-1.42)	40	0.52±0.16 (0.21-1.07)	0.01
GOT (IU/*l*)	42	24.1±7.0 (15-59)	37	342.5±382.0 (51-1681)	0.01
GPT(IU/*l*)	39	22.9±5.3 (13-40)	40	245.7±335.0 (41-1601)	0.01
Creatinine (mg/d*l*)		ND[d)]	14	3.01±2.20 (1.45-8.69)	—
Bilirubin (mg/d*l*)		ND	38	1.91±2.20 (0.15-8.20)	—
Plasma hemoglobin (mg/d*l*)	20	3±2 (1-8)	38	161±181 (11-954)	0.01

a) Standard deviation. b) Probability of significant difference. c) Range. d) Not done.

Table 2. Levels of measured and calculated serum osmolalities and osmolar gap in the control and hemoglobinuria groups

Parameter	Control		Hemoglobinuria[b)] Recovered cases		Hemoglobinuria[b)] Fatal cases	
	n	Mean±SD[a)]	n	Mean±SD	n	Mean±SD
Measured osmolality (mOsm/kg)	43	296±5 (289-308)[c)]	29	302±17 (272-347)	11	331±28** (298-370)
Calculated osmolality (mOsm/kg)	19	293±6 (281-311)	24	296±16 (271-333)	10	304±22 (277-333)
Osmolar gap (mOsm/kg)	19	1.5±4.9 (−4-11)	24	5.4±5.9 (−6-18)	10	22.7±8.9** (8-37)

a) Standard deviation. b) Pre-operative values. c) Range.
**: Statistically significant difference from the control group and recovered cases ($p<0.01$).

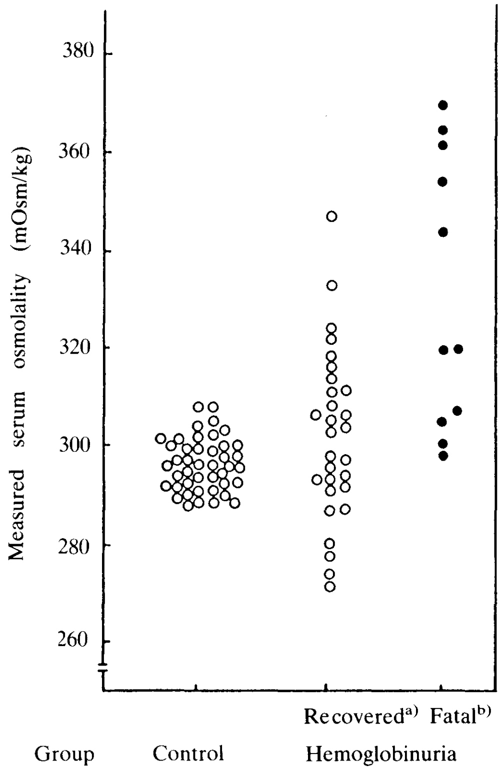

Fig. 1. Measured serum osmolality in the control and hemoglobinuria groups
a) Cases that recovered after heartworm removal.
b) Cases that died after heartworm removal.

afterwards had osmolar gaps falling in the normal range, while in the cases of death, the values obtained went over that range.

The mean values of osmotically active substances in serum are shown in Table 3. Serum sodium level ranged from 132.0 to 159.5 mEq/*l*, and its control level was comparable to those of the recovered and fatal cases in the hemoglobinuria group. The serum potassium levels also were in the same range among them. In the hemoglobinuria group, the BUN values varied greatly, from normal value to extremely high (152.0 mg/d*l*). The mean value was the highest in the fatal cases, and that of recovered cases came to the next. The serum glucose level was significantly higher in recovered cases than in the controls or fatal cases.

The relationships between the measured osmolality or osmolar gap and other test results before surgery in the hemoglobinuria group are shown in Table 4. Measured osmolality correlated significantly with the osmolar gap, sodium, potassium, BUN, GOT, GPT, creatinine, bilirubin and plasma hemoglobin, while the osmolar gap correlated significantly with potassium, BUN, GOT, GPT, creatinine and bilirubin.

The levels of plasma hemoglobin, serum osmolalities and osmotically active substances before and 20 hr after the surgical removal of heartworms are shown in Table 5. The plasma hemoglobin levels fell markedly following the treatment in both the recovered and almost all death cases. They did not, however, show significant changes in the measured osmolality, osmolar gap, or sodium levels in both cases after the heartworm removal, whereas the calculated osmolality, potassium, BUN and glucose levels significantly decreased, exclusively in the recovered cases. However,

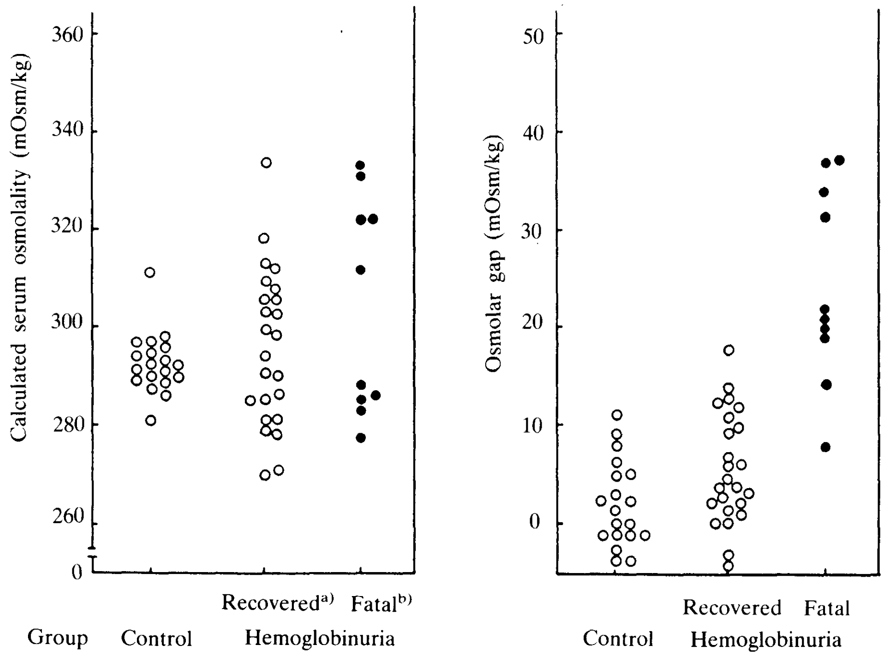

Fig. 2. Calculated serum osmolality and osmolar gap in the control and hemoglobinuria groups
a) Cases that recovered after heartworm removal.
b) Cases that died after heartworm removal.

Table 3. Concentrations of serum osmotically active substances

Item	Control		Hemoglobinuria				p<[b)]
			Recovered cases		Fatal cases		
	n	Mean±SD[a)]	n	Mean±SD	n	Mean±SD	
Sodium (mEq/l)	19	149.7±3.0 (143.2-158.0)[c)]	24	144.1±7.1 (132.0-159.5)	10	144.5±8.2 (132.0-158.2)	NS[d)]
Potassium (mEq/l)	19	4.46±0.42 (3.81-5.50)	18	4.72±1.00 (3.00-6.80)	10	4.48±1.48 (2.10-6.55)	NS
BUN (mg/dl)	42	15.6±3.0 (8.9-20.5)	29	46.0±28.2**[e)] (10.0-112.9)	11	83.5±45.0** (13.8-152.0)	0.01
Glucose (mg/dl)	42	85.3±9.9 (67.0-104.2)	29	117.2±35.9** (71.3-203.0)	11	85.4±32.2 (46.3-139.0)	0.01

a) Standard deviation. b) Probability of significant difference between the recovered and fatal cases.
c) Range. d) Not significant. e) Probability of significant difference against the control group with $p<0.01$.

Table 4. Correlations between the measured serum osmolality or osmolar gap and other measurement items in the hemoglobinuria group

Item	Measured osmolality			Osmolar gap		
	n	r[a)]	p<[b)]	n	r	p<
Osmolar gap	38	0.71	0.01		—	
Sodium	38	0.41	0.05	38	−0.03	NS[c)]
Potassium	38	0.41	0.05	38	0.52	0.01
BUN	40	0.71	0.01	38	0.47	0.01
Glucose	40	−0.16	NS	38	−0.25	NS
A/G ratio	40	−0.10	NS	38	−0.25	NS
GOT	35	0.57	0.01	35	0.47	0.01
GPT	35	0.35	0.05	35	0.43	0.01
Creatinine	14	0.79	0.01	14	0.93	0.01
Bilirubin	38	0.55	0.01	30	0.42	0.05
Plasma hemoglobin	38	0.40	0.05	30	0.17	NS

a) Correlation coefficient. b) Probability of significant correlation coefficient. c) Not significant.

Table 5. Levels of plasma hemoglobin, serum osmolalities and osmotically active substances before and 20 hr after heartworm removal in the hemoglobinuria group

Item	Recovered cases				Fatal cases			
		Before[a)]	After[b)]	p<[c)]		Before	After	p<
	n	Mean±SD[d)]	Mean±SD		n	Mean±SD	Mean±SD	
Plasma hemoglobin (mg/d*l*)	28	119±98	6±4	0.01	7	224±328	47±104	NS[e)]
Measured osmolality (mOsm/kg)	29	302±17	298±16	NS	7	327±32	327±37	NS
Calculated osmolality (mOsm/kg)	20	295±16	288±17	0.01	7	303±24	302±30	NS
Osmolar gap (mOsm/kg)	20	5.8±6.1	7.9±7.7	NS	7	21.6±6.1	22.1±10.6	NS
Sodium (mEq/*l*)	20	143.5±7.1	142.8±6.8	NS	7	142.6±6.9	142.6±7.4	NS
Potassium (mEq/*l*)	13	4.81±1.14	4.15±0.66	0.05	7	4.33±1.73	5.03±2.07	NS
BUN (mg/d*l*)	29	46.0±28.2	31.7±23.9	0.01	7	76.9±45.8	73.1±56.2	NS
Glucose (mg/d*l*)	29	117.2±35.9	100.9±26.5	0.01	7	97.4±34.3	100.2±45.2	NS

a) Before heartworm removal. b) 20 hr after heartworm removal. c) Probability of significant difference. d) Standard deviation. e) Not significant.

serum osmolalities and such osmotically active substances did not change significantly after surgery in cases that dies afterwards.

DISCUSSION

Measured serum osmolalities in normal dogs have been reported as 280–310 mOsm/kg [22], 266–300 mOsm/kg (mean was 286 mOsm/kg) [4] or 296±9 mOsm/kg [9]. In the present study, measured serum osmolality in the control group ranged from 289 to 309 mOsm/kg with a mean of 296±5 mOsm/kg, in agreement with the data from other investigators. Among the many formulas for the calculation of osmolality [6, 10], the formula of Boyd *et al.* [3] was employed in the present study, because the osmolar gap in the control group was minimal. According to this formula, the osmolar gap in the control group was calculated as 10 mOsm/kg or less.

In the hemoglobinuria group, the measured serum osmolality ranged widely, and a considerable number of dogs evidenced normal serum osmolalities in spite of severe intravascular hemolysis. Therefore, the changes in serum osmolality would not be the cause of intravascular hemolysis. Widespread values in serum osmolality indicated various disorders involving the serum osmolality. The measured serum osmolality correlated significantly with the serum sodium and potassium concentrations. However, the serum sodium level tended to decrease in association with a small amount of potassium gain in the hemoglobinuria group. Thus, these electrolytes could not contribute to the increase in measured serum osmolality in the hemoglobinuria group. Moreover, the measured osmolality correlated significantly with osmolar gap, BUN, creatinine, bilirubin, GOT and GPT in the hemoglobinuria group. The highly significant correlation between the measured serum osmolality and BUN or creatinine suggested that the renal function was closely concerned with serum osmolality. In renal failure, many osmotically active substances such as urea, creatinine, guanidine compounds, ammonia and unknown uremic toxins stagnate in the circulatory system, raising serum osmolality. Besides renal dysfunction, the disease accompanies severe circulatory disturbances, intravascular hemolysis, dehydration and injuries of the various organs [12, 14, 15, 17]. Under these conditions, large amounts of various abnormal substances, which are osmotically active, might be released from the injured cells or organs and stagnate in the circulatory system. Higher serum osmolalities and osmolar gaps found in the hemoglobinuria group may reflect the degree of organ injuries.

The large osmolar gap, which implies an increase in a number of unmeasured abnormal substances other than sodium, urea and glucose, is found at the terminal phase or shock-like state, reflecting very poor prognosis [1, 2, 5, 9, 18, 21]. Also in the present study, the fatal cases tended to have large osmolar gaps, suggesting a near-critical situation. The determination of serum osmolality and osmolar gap may have a clinical significance for diagnosis and prognosis in dogs with dirofilarial hemoglobinuria.

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要　約

犬糸状虫性血色素尿症に関する研究，実測および算出血清浸透圧ギャップ：北川　均・佐々木栄英・石原勝也（岐阜大学農学部家畜内科学講座）——健康ビーグル43頭（対照群）と犬糸状虫性血色素尿症自然例40頭（血色素尿症群）について血清浸透圧を測定するとともに算出浸透圧と浸透圧ギャップを算定した．対照群では，血清の実測浸透圧は296±5 mOsm/kg，算出浸透圧は293±6 mOsm/kg，浸透圧ギャップは10 mOsm/kg 以下であった．血色素尿症群の実測浸透圧は272から370 mOsm/kg の広い範囲に分布していた．しかし，かなりの例数が激しい血管内溶血にもかかわらず正常範囲内の血清浸透圧を示し，血清浸透圧の変化は溶血の直接的原因ではなかった．犬糸状虫摘出手術後死亡した11例の実測浸透圧（331±28 mOsm/kg）は，回復した29例（302± 17 mOsm/kg）より高値であった．算出浸透圧は回復24例では296±16 mOsm/kg，死亡18例では304±22 mOsm/kg であった．摘出後回復した例の浸透圧ギャップは，正常範囲内に分布する傾向を認めた（5.4±5.9 mOsm/kg）が，死亡例では正常範囲より高い値を示し（22.7±8.9 mOsm/kg），浸透圧ギャップは予後を示唆した．実測浸透圧は，浸透圧ギャップ，ナトリウム，カリウム，BUN，GOT，GPT，クレアチニン，ビリルビンおよび血漿ヘモグロビン濃度等と有意に相関した．Osmolar gap は，カリウム，BUN，GOT，GPT，クレアチニンおよびビリルビン濃度と有意に相関した．犬糸状虫摘出20時間後，血漿ヘモグロビン濃度は著しく下降したが，血清浸透圧には明瞭な変化がなかった．