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Research Article

ARGININE STATUS IN DOGS WITH PERSISTENT HYPERAMMONEMIA

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ABSTRACT

Hyperammonemia is a medical condition marked by excessive levels of ammonia in the blood and includes a variety of symptoms indicative of significant CNS abnormalities. Twenty one dogs of various age group and breed were presented at Teaching Veterinary Clinical Complex, Mumbai Veterinary College, Parel with hyperammonemic symptoms like seizures, wobbling, paddling, altered pupilary light reflex (PLR), hyperthermia, GIT bleeding, nystagmus, vomition, delirium, aggressive behavior, compulsive pacing, head pressing, ataxia and sudden collapse. The dogs were treated for hyperammonemia with L-ornithine-L-aspartate (LOLA), Gutacting antibiotic (Ampicillin cloxacillin combination), multivitamin preparation, metronidazole and restricting dietary protein intake. Based on response to treatment, the dogs were classified into two groups: group A (persistent hyperammonemic) and group B (responsive to treatment). Persistently hyperammonemic dogs were further subjected to serum bile acids and quantitative plasma amino acid analysis to rule out arginine deficiency and acquired portosystemic shunt. In Group A (persistent hyperammonemic), 33.33% (n=5) cases showed arginine deficiency while in Group B (Responsive to treatment) 20% (n=1) of cases were found to be deficient in arginine. The dogs with arginine deficiency responded well to arginine treatment because of enhanced nitrogen excretion and restricted protein catabolism. The study concluded that, in addition to a reduced protein consumption, arginine supplementation is required to lower the blood ammonia levels and improve clinical symptoms in dogs with persistent hyperammonemia.

Keywords: canine, healthy dogs, HPLC, plasma free arginine

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Hyperammonemia is a medical disorder characterized by high amounts of ammonia in the blood that is manifested with various central nervous system (CNS) abnormalities (Auron and Brophy, 2012). Hyperammonemia can be caused by a variety of factors: portosystemic shunt (Tivers *et al.*, 2014), hepatic insufficiency (Albrecht and Dolinska, 2001), decreased urea cycle enzyme activity (Lanpher *et al.*, 1993) and organic acid metabolic diseases (Kasapkara *et al.*, 2011).

The most common cause of hyperammonemia is inherited urea cycle enzymatic defects. The greater the degree of hyperammonemia, the closer the enzyme deficiency is to the point of ammonia entrance into the urea cycle (Flannery *et al.*, 1982). Primary urea cycle disorders (UCDs) including carbamoyl phosphate synthase (CPS) deficiency, ornithine transcarbamylase (OTC) deficiency, arginino-succinate synthetase deficiency (citrullinemia), arginino succinate lyase deficiency (arginino-succinic aciduria) and arginase deficiency (argininemia).

Arginine is necessary for urea cycle function and is a quasi-essential amino acid when a defect in the urea cycle is present (Clay and Hainline, 2007). Ammonia elimination from the blood is facilitated by arginine and the Krebs urea cycle. Correction of an arginine shortage in a poorly nourished patient with a high ammonia level is critical (Fahey *et al.*, 1957). There are three conditions that can cause arginine deficiency: dietary deficiency of arginine, which can be caused by starvation or consuming a diet that is severely deficient in arginine, increased catabolism of arginine, which is usually caused by arginase, and decreased rate of endogenous arginine synthesis (Morris, 2012). The present study was aimed to establish a relationship between the arginine levels in dogs having persistent hyperammonemia and to therapeutic manage the deficiency by feeding arginine.

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MATERIALS AND METHODS

Twenty one dogs of various age group and breed presented at Teaching Veterinary Clinical Complex, Mumbai Veterinary College, Parel with hyperammonemic symptoms like neurological signs such as seizures, wobbling, paddling, altered pupillary light reflex (PLR), delirium, aggressive behavior, compulsive pacing, head pressing, ataxia, sudden collapse and other signs like vomiting, gastrointestinal bleeding (GIT bleeding) and hyperthermia were subjected to Complete blood count (CBC)estimated by Penta 5 part Blood Analyser, liver & kidney function estimated by Semi Automated analyzer ARX-100 and Fully Automated analyzer FA-200.

Plasma ammonia tests determined by Dry Chemistry to rule out hepatic involvement of hyperammonemia

Based on results of above tests, the dogs found to have a high plasma ammonia level (≥ 86 µg/dL) were

treated for hyperammonemia with L-ornithine-L-aspartate (LOLA) 0.5 g/kg b.wt. IV diluted in Dextrose 5%, gut acting antibiotic Inj. Ampicillin + Cloxacillin @ 10 mg/kg b.wt. IV, Inj. multivitamin, Metronidazole 5-10 mg/kg b.wt. orally and restricting dietary protein intake. The parameters recorded before and after treatment is presented in Table 1 and 2.

Inspite of the above treatment, 16 dogs out of 21 selected dogs showed persistent hyperammonemia *i.e.* an elevated plasma ammonia even after 3 days of therapy and had no improvement in the clinical signs were categorized as persistently hyperammonemic dogs (Group A) and five were responsive cases (Group B). These groups were further subjected to serum bile acids and quantitative plasma amino acid analysis to rule out arginine deficiency and acquired portosystemic shunt. Firstly, the objective was to identify and separate the persistant hyperammonemia cases and secondly, a comparison was done for the arginine levels in persistant cases with that of the responsive cases.

Quantitative plasma amino acid analysis was performed by High Performance Liquid Chromatography (HPLC) technique to estimate the levels of free amino acid arginine (Azuma *et al.*, 2016). Plasma samples were collected after an 8 hour fast to minimize dietary influences on circulating amino acids and was deproteinized with 400 µl methanol (plasma: methanol (v/v)=1:4) in 1.5 ml eppendorf tube. The mixture was vortex mixed for 3 min and kept overnight at -20 ºC and centrifuged for 10 min at 15,000 rpm to remove the precipitated proteins. 200 µl of the supernatant was dried in a Speed Vacconcentrator. The dried residues were dissolved in 50 µl of 0.1N HCl and then 1 ìl of the mixture supernatant was subjected to pre column derivatization method using an Agilent 1290 series UHPLC system with a binary pump delivery system coupled to a Agilent 1260 Infinity Diode Array Detector.

The Mean and standard error of collected data was calculated and analysed for comparison as per methods suggested by Snedecor and Cochran (2002).

RESULTS AND DISCUSSION

The Mean±SE values of CBC of selected dogs (n=21) before and after treatment were recorded and is presented in Table 1. Significant (P≤ 0.05) decrease was observed in the TLC and Neutrophil count after treatment. Difference in other CBC parameters were non-significant (P≤ 0.05). The Mean ± SE values of LFT, KFT and Plasma ammonia are presented in Table 2. No significant (P≤ 0.05) changes were observed in liver and kidney function parameters before and after treatment. The Plasma Ammonia was 125.85 ± 8.78 µg/dl initially, that significantly decreased (P ≤ 0.05) to 91.90 ± 6.41µg/dl after treatment. Administration of L-Ornithine L-Aspartate (LOLA) led to significant lowering of Plasma Ammonia and resolution of clinical signs in 5 cases which is in agreement with findings of Ahn et al. (2016).

Inspite of the above treatment, 16 dogs out of 21 selected dogs showed Persistent Hyperammonemia *i.e.* showed an elevated Plasma Ammonia even after 3 days of therapy and had no improvement in the clinical signs. These dogs were categorized as Group A and five dogs who responded to the above therapy were categorized as Group B. Mean ± SE of Arginine was 89.83 ± 11.29 nmol/ml and 84.28 ± 20.66 nmol/ml in Group A and Group B dogs, respectively. In Group A, 33.33% (n=5) cases showed arginine deficiencies arginine ranging from 29.37 nmol/ml to 53.99 nmol/ml, while 20% (n=1) of cases in Group B was found to be deficient in arginine *i.e.* 22.34 nmol/ml. The normal level of amino acid arginine in healthy dog ranges from 64.8 to 165.9 nmol/ml (Chan *et al.*, 2009). In comparison to group A, arginine values are lower in group B, implying that arginine levels have no bearing on initial therapeutic responsiveness.

Urea cycle disorders are characterised by low arginine levels and recurrent hyperammonemia. Batshaw (1984), Lo *et al.* (1993) and Lanpher *et al.* (1993) observed that the influence of an enzymatic defect in the urea cycle causes arginine to become a necessary amino acid (except in arginase deficiency) and waste nitrogen to accumulate primarily as ammonia.

The findings were also in agreement with Machado and Silva (2014) who observed that the plasma concentrations of arginine are reduced in every type of UCD like carbamoyl phosphate synthase I deficiency (CPS 1 deficiency), ornithine transcarbamylase (OTC deficiency), arginino- succinate synthetase 1 (ASS-1 deficiency), arginino-succinate lyase (ASL deficiency), except in arginase deficiency (elevation five to sevenfold). In late onset or in partial enzyme defects, arginine concentrations may be normal.

Among the selected dogs (n=21), arginine deficiency with seizure was observed in 24% (n=5/21) of cases and arginine deficiency without seizure was observed in 4.76% (n=1/21) (yes group B dog) of cases. This observation was similar to Tivers *et al.* (2014) who reported dogs with urea cycle enzyme deficiency with increased plasma ammonia concentrations but did not typically developing clinical signs of HE.

The absolute arginine need varies depending on the dog's age and the protein source. Arginine is a dietary essential amino acid for the mature dog, and 0.28 percent

Table 1

Summary of Mean ± SE of complete blood count of selected dogs (n=21) before and after treatment along with paired t-test

S.No.	Parameter	Before Treatment (n= 21) Mean ± S.E.	After Treatment (n= 21) Mean ± S.E.	t-value (df = 20)	t-table
1	Hb (gm%)	12.34 ± 0.79	12.35 ± 0.81	0.05	2.08
2	PCV (%)	37.27 ± 2.37	37.38 ± 2.31	0.30	
3	TEC (x 106/cmm)	5.52 ± 0.35	5.28 ± 0.32	2.01	
4	MCV (fl)	67.52 ± 1.03	67.02 ± 1.17	1.19	
5	MCH (pg)	22.3 ± 0.47	21.86 ± 0.47	1.45	
6	MCHC (g/dl)	33.03 ± 0.45	32.36 ± 0.71	1.25	
7	TLC (/cumm)	15981 ± 1346.61	13695.1 ± 665.97	2.57*	
8	N (%)	75.04 ± 1.93	71.76 ± 1.13	2.41*	
9	E (%)	1.33 ± 0.33	1.38 ± 0.29	0.12	
10	L (%)	21.23 ± 1.90	25.76 ± 1.11	1.27	
11	M (%)	1.38 ± 0.17	1.09 ± 0.16	1.61	
12	B (%)	0	0	-	
13	PLT (lacs /cumm)	257714 ±19425.9	258357 ± 16577.1	0.10	

Means to be read column wise. ($p<0.05$), *significant

Table 2

Summary of Mean ± SE of biochemical tests of selected dogs (n=21) before and after treatment along with paired t-test

S.No.	Parameter	Before Treatment (n= 21) Mean ± S.E.	After Treatment (n= 21) Mean ± S.E.	t-value (df = 20)	t-table
1	TB (mg/dl)	0.46 ± 0.03	0.43 ± 0.01	1.93	2.08
2	DB (mg/dl)	0.19 ± 0.01	0.19 ± 0.01	0.32	
3	IB (mg/dl)	0.26 ± 0.02	0.23 ± 0.01	0.50	
4	SGOT (IU/dl)	75.02 ± 28.99	68.85 ± 19.52	0.17	
5	SGPT (IU/dl)	167.14 ± 96.32	96.95 ± 44.27	0.64	
6	ALP (IU/L)	212.59 ± 38.69	198.73 ± 35.92	0.26	
7	TP (gm/dl)	6.91 ± 0.27	6.77 ± 0.16	0.69	
8	Albumin (gm/dl)	2.53 ± 0.09	2.74 ± 0.11	2.06	
9	Globulin (gm/dl)	4.30 ± 0.25	4.02 ± 0.17	1.05	
10	A/G ratio	0.62 ± 0.04	0.71 ± 0.05	1.55	
11	BUN (mg/dl)	15.18 ± 1.59	16.91 ± 0.64	1.13	
12	Creatinine (mg/dl)	0.98 ± 0.06	0.91 ± 0.05	0.12	
13	Plasma Ammonia (µg/dl)	125.85 ± 8.78	81.33 ± 5.10	7.44*	

Means to be read column wise. ($p<0.05$), *significant

dietary arginine prevents major changes in the mature dog's normal intermediate metabolism (Burns *et al.*, 1981).

In patients with diseases where in vivo arginine production is limited by enzyme deficiencies, intravenous arginine treatment may also improve nitrogen excretion by limiting protein catabolism (Brusilow, 1984).

CONCLUSION

From the above study it can be concluded that to reduce ammonia levels from the blood along with reduced protein intake supplementation of arginine should be given to relieve clinical symptoms of suffering dogs with hyper-ammonemia.

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