RESEARCH ARTICLE

COMPARATIVE 1H-NMR-BASED CEREBROSPINAL AMINO ACID PROFILING IN TICK-PARALYZED AND HEALTHY DOGS

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ABSTRACT

Tick paralysis, caused by neurotoxins released by certain tick species during blood feeding, leads to ascending acute flaccid paralysis (AFP) and can result in severe complications, such as respiratory failure and death. Metabolomic profiling of amino acids, particularly using 1H-NMR, is a valuable tool for understanding the mechanisms underlying these conditions. In this study, 92 dogs presenting with clinical signs, including sudden onset of weakness, difficulty moving, and hind limb incoordination indicative of AFP, were evaluated at the Harran University Veterinary Faculty Animal Hospital. 15 dogs were assigned to the Paralysis group and 10 to the Healthy group based on their respective inclusion/exclusion criteria. CSF samples were collected from all dogs, and 1H-NMR-based amino acid profiling was performed on all samples using an Agilent 400 MHz spectrometer. The Paralysis group exhibited higher body temperature, heart rate, and respiratory rate compared to the Healthy group ($p < 0.028$). Paralyzed dogs had a shorter capillary refill time ($p < 0.008$), while healthy dogs had a higher Modified Glasgow Coma Scale (MGCS) score. Regarding amino acid concentrations, the Paralysis group had higher levels of L-phenylalanine, L-isoleucine, L-histidine, lysine, and L-tryptophan ($p < 0.038$), and lower levels of L-threonine, L-leucine, L-methionine, and L-valine ($p < 0.036$). These findings suggest that the increased levels of certain amino acids reflect a neuroprotective response to neuroinflammation, while the decreased levels point to neuronal damage and disrupted transfer mechanisms. Overall, this study enhances the understanding of tick paralysis and may provide insights into other non-infectious AFP conditions.

Keywords: Acute flaccid paralysis, biomarker, dog, tick paralysis

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INTRODUCTION

Certain tick species, such as *Ixodes holocyclus* and *Rhipicephalus sanguineus* (*R. sanguineus*), the most widespread tick of dogs and also known as the brown dog tick, can release neurotoxins through their salivary glands during blood feeding, causing tick paralysis (Otranto et al., 2012). This condition, with tick paralysis as a part of its etiology, is characterized by rapid, progressive acute flaccid paralysis (AFP) and can be life-threatening (Hogan et al., 2019; Gülersoy et al., 2024a). Clinical signs typically begin with weakness and incoordination, progressing to paralysis. Affected dogs may experience difficulty breathing, gagging, coughing, and, in severe cases, respiratory failure and death can occur. Severity of symptoms depends on factors like tick size, attachment duration, and the dog's sensitivity to the toxin (Hogan et al., 2019).

Diagnosis is based on clinical signs and recent tick exposure, supported by identifying ticks on the dog or in its environment. Laboratory tests, including complete blood count (CBC), serum biochemistry, and cerebrospinal fluid (CSF) analysis, help assess neurological function and exclude other conditions (Mackenzie, 2011; Otranto et al., 2012). However, in cases of tick paralysis, CSF analysis findings—typically evaluating parameters such as glucose or total leukocytes—may remain within reference values due to non-infectious nature of tick paralysis (Hogan et al., 2019). Metabolomic profiling of amino acids is a promising approach for uncovering underlying mechanisms, identifying biomarkers, and informing treatment strategies for autoimmune and neurodegenerative diseases (Gülersoy et al., 2024b). Under normal conditions, amino acid concentrations in CSF are significantly lower than in plasma. Minor disturbances in the CSF amino acid profile have been reported in neurological diseases such as Parkinsonism, epilepsy, and Huntington's chorea (Link and Tibbling, 1977). Guillain-Barré syndrome, an acute flaccid paralysis similar to tick paralysis, is also associated with elevated CSF protein concentrations at certain stages, which are believed to result largely from blood-CSF barrier disruption (Hegen et al., 2021).

Promising results have been reported in 1H-NMR-based metabolomics studies of tick salivary neurotoxin in serum samples from dogs with tick paralysis (Simon et al., 2023; Gülersoy et al., 2024c). 1H-NMR-based CSF amino acid profiling may also be a valuable tool for evaluating different stages of the disease. Therefore, this study aims to analyze 1H-NMR-based amino acid profiles in CSF samples from dogs with tick paralysis, investigate potential diagnostic and prognostic markers, and provide insights for further acute flaccid paralysis studies.

MATERIAL AND METHODS

This study was approved by the Local Ethics Committee for Animal Experiments at Harran University (Date: 09.05.2022.; Decision Number: 2022/003–01/06). In addition, informed consent was obtained from all dog owners prior to the commencement of the study. No experimental procedures that could harm the animals or compromise their welfare were conducted.

Animals

Both the paralyzed and healthy animals in this study were selected from 92 dogs admitted to Harran University Veterinary Faculty Animal Hospital between January and October, 2024. Among these dogs, those without any comorbid diseases, as determined by physical and laboratory examinations, and with findings suggestive of AFP, were included in the Paralysis group. Dogs that were determined to be healthy based on physical and laboratory examinations and were admitted for vaccination or routine check-ups were included in the Healthy group.

Physical Examinations and Inclusion/Exclusion Criteria

During the physical examination, body weight, body temperature, heart rate, respiratory rate, and gingival capillary refill time (CRT) were assessed, and Modified Glasgow Coma Scale (MGCS) scores were calculated for all dogs. Tick-paralyzed

dogs were examined for ticks by thumb-counting across anatomical regions, including the head, neck, ears, thorax, abdomen, interdigital areas, forelimbs, hind limbs, tail, axillary, and inguinal regions. Morphological examination of ticks collected from paralyzed dogs was performed by an expert for species identification, and they were identified as *R. sanguineus*.

Inclusion criteria for the study required that the dog had no history of any disease, had not received antiparasitic medication recently (<1 month), had an engorged tick, and displayed signs of AFP. Clinical findings suggestive of AFP included an inability to contract due to motor pathway impairment from the cortex to muscle fibers, absence of spasticity or other signs of disordered central nervous system motor tracts (e.g., hyperreflexia, clonus, or extensor plantar responses), and the sudden onset and progression of weakness, particularly affecting respiratory muscles and swallowing (Growdon and Fink, 1994; Marx et al., 2000). To avoid interfering with the NMR-based CSF amino acid profiling, CBC and microscopic blood smear examinations were performed on all dogs included in the study. Blood and buffy coat smears were examined for Anaplasma platys, Ehrlichia canis, Babesia spp., and Hepatozoon canis inclusions. Each smear was analyzed under a 100× oil immersion objective to ensure optimal morphology using a light microscope. Dogs with blood parasites or abnormalities such as thrombocytopenia or pancytopenia, commonly observed in dogs previously infected with *R. sanguineus* (Otranto et al., 2012), were excluded from the study. Additionally, CBC results, including leukogram and hemogram indices, were used solely as inclusion/exclusion criteria and were not evaluated further within the scope of this study.

Forming Subgroups

15 dogs infested with *R. sanguineus*, with clinical findings compatible with AFP due to tick infestation and with a confirmed tick paralysis diagnosis ex juvantibus, constituted the Paralysis Group of the study. 10 dogs with similar body weights and similar ages ($p < 0.580$), which were admitted either for vaccination and/or check-up purposes, constituted the healthy Control Group.

CSF Sampling

Before cerebrospinal fluid tap, all tick paralyzed dogs were sedated by intramuscular injection at a dose of 1 mg / kg with xylazine (Xylazin Bio® 2%, Bioveta) after blood sampling. CSF samples were taken (1–2 mL) between the occipital and atlas bones with the appropriate procedure (using a 22 gauge, 1.5 inch stylet spinal needle) (Gülersoy et al., 2024b). Excessive flexion of the head was avoided to prevent airway obstruction. There were no complications observed during and after the procedure.

NMR Analysis

CSF samples were prepared as previously reported (Gülersoy et al., 2024b). The 1H-NMR spectra were acquired at 26.5 °C using an Agilent 400 MHz spectrometer operating at 400.13 MHz, equipped with a 5 mm inverse detection probe with z-axis gradients. The samples were placed in 5 mm Wilmad 507 NMR tubes. Spectral data were recorded using the NOESY presaturation pulse sequence, with the following parameters: 32 scans, a 30 s relaxation delay, 4 s acquisition time, 8223 Hz spectral window, and 64 K data points, providing a digital resolution of 0.12 Hz. The Free Induction Decay (FID) was processed with an exponential line broadening factor of 0.3 Hz. Chemical shifts are reported in δ values (ppm), with TSP (0.0 ppm) as the internal reference. Acquisition and data processing were carried out using Agilent SpectrAA software. The NMR protocol ensured complete signal relaxation with an overall relaxation delay of 37 s. The combination of 0.3 Hz line broadening and 37 s relaxation delay allows for the use of signal intensities in quantification, reducing errors from partial signal overlap in crowded spectra. For each NMR measurement, 600 µL of sample (the standard 5 mm NMR tube volume) was used, after reducing the CSF sample to the minimum required volume. Using the full filling volume for one measurement yields better results compared

to two half-volume measurements, as it improves shimming, resulting in a higher signal-to-noise ratio and increased sensitivity (enhanced magnetic field homogeneity, leading to sharper spectral lines). All sample preparations and measurements were performed by a single operator to minimize operator-induced variability. Analytical error was found to be significantly lower than biological variation when the same operator conducted the tests. For four samples prepared from the same batch, the analytical error for various amino acids ranged from 1-5%, while the biological variation (expressed as % RSD) varied between 10-45%.

Identification and Quantification of CSF Amino Acids

The identification and quantification of CSF amino acids from raw 1D NMR spectra (FID files) acquired on the Agilent spectrometer were carried out using BAYESIL software. BAYESIL provides fully automated spectral processing and profiling for 1D and 1H-NMR spectra, regardless of the acquisition frequency, and is compatible with standard NMR instruments. During spectral deconvolution, BAYESIL divides the spectrum into smaller segments and employs a probabilistic graphical model to represent the sparse dependencies between these segments. Approximate inference is then applied to this model, effectively serving as a stand-in for spectral profiling, leading to the most probable amino acid profile. BAYESIL facilitates a range of spectral processing functions, including zero filling, phasing, baseline correction, smoothing, chemical shift referencing, and reference deconvolution, starting with the raw spectrum. The relative concentrations of the quantified amino acids were determined based on the total area of the spectrum. Statistical analysis of the relative concentrations was also performed using BAYESIL. Visualization, simulation, and presentation of the NMR spectra were carried out using MestReNova software (MestreLab Research, Spain).

Statistical Analysis

Data analysis was performed using SPSS 25.00 (SPSS for Windows®). To assess whether the data followed a parametric or non-parametric distribution, a one-sample Kolmogorov-Smirnov test was conducted. Since the data were determined to be non-parametric, they were analyzed as median (min-max) values using the Mann-Whitney U and Kruskal-Wallis tests. Statistical significance was defined as $p < 0.05$.

RESULTS

Animals

All dogs in the present study were domestic, unvaccinated, fed commercial dry dog food, and taken outside for walks 2-3 times a day. Most dogs in the Paralysis group were housed in rural areas (11 out of 15 dogs). In contrast, all dogs in the Healthy group were housed in more urbanized areas (10 out of 10 dogs). Paralyzed dogs exhibited neurological findings sufficient to raise suspicion of AFP, such as sudden onset of weakness, difficulty in movement, hind limb incoordination, or quadriplegia. Anamnestic data revealed that the dogs had no history of disease previously. The symptom duration of tick paralyzed dogs was 3 (2-6) days. These dogs had ticks detected during clinical examination and were admitted for diagnosis and treatment. The physical examination and CBC findings of dogs classified as healthy were within normal limits. Of the 92 dogs evaluated based on all examinations and the specific inclusion/exclusion criteria of each group in this study, 15 were assigned to the Paralysis group and 10 to the Healthy group.

Physical Examination Findings

A thorough inspection revealed a median of 33 ticks (range: 10–65) in the tick-paralyzed dogs. Some owners had attempted to remove a few ticks on their own prior to the dogs' arrival at the hospital. Additional ticks were removed during the clinical examination. To confirm the ex juvantibus diagnostic approach, all dogs were treated with a spot-on formulation containing Fipronil 10% / (S)-Methoprene 9% (Frontline Combo, Merial S.A.S., France). Compared to the Healthy group, the Paralysis group exhibited higher body temperature, heart rate, and respiratory rate values ($p < 0.028$).

The CRT was shorter in paralyzed dogs ($p < 0.008$), while the MGCS score was higher in the healthy dogs. Demographic data and physical examination findings are presented in Table 1.

Table 1 Physical examination findings

Parameters	Paralysis Group n:15 median (min-max)	Healthy Group n:10 median (min-max)	p value
Body temperature (°C)	39.6 (38.7-40.5)	38.1 (37.7-38.5)	0.0001
Heart rate (beats/min)	104 (90-152)	78 (65-96)	0.0001
Respiratory rate (breaths/min)	54 (40-68)	42 (33-55)	0.009
CRT (sec)	1 (1-2)	2 (1-3)	0.001
MGCS	6 (3-13)	16 (15-18)	0.0001
Body weight (kg)	6.22 (4.05-8.15)	6.18 (4.35-8.11)	0.769
Age (months)	5 (4-6.5)	5.75 (5.5-6.5)	0.457

CRT: Capillary Refill Time, MGCS: Modified Glasgow Coma Scale.

1H-NMR-based CSF Amino Acid Profiling Results

Compared to the Healthy group, the Paralysis group had higher concentrations of L-phenylalanine, L-isoleucine, L-histidine, lysine, and L-tryptophan ($p < 0.038$), and lower concentrations of L-threonine, L-leucine, L-methionine, and L-valine ($p < 0.036$). The CSF amino acid profiling results are presented in Table 2. The identified and quantified metabolites are visualized in Figure 1 and 2.

Table 2 1H-NMR-based CSF Amino Acid Profiling

Parameters*	Paralysis Group n:15 median (min-max)	Healthy Group n:10 median (min-max)	p value
L-phenylalanine	4.72 (0.11-8.44)	0.22 (0.13-0.37)	0.017
L-threonine	14.35 (10.2-69.9)	33.69 (18.1-50.98)	0.036
L-isoleucine	2.32 (1.33-3.92)	1.73 (0.27-3.88)	0.055
L-histidine	4.65 (0.1-7.1)	0.5 (0.2-0.8)	0.010
Lysine	35.56 (14.6-57.61)	29.98 (24.73-36.74)	0.038

Parameters*	Paralysis Group n:15 median (min-max)	Healthy Group n:10 median (min-max)	p value
L-leucine	5.5 (3.8-8.7)	19.03 (7.71-39.95)	0.016
L-methionine	39.56 (15.45-65.14)	215.73 (56.41-272.03)	0.001
L-valine	7.7 (6.2-11.4)	26.97 (15.2-43.11)	0.004
L-tryptophan	2.8 (1.4-4.1)	0.29 (0.14-0.64)	0.0001

*Concentrations are in arbitrary units

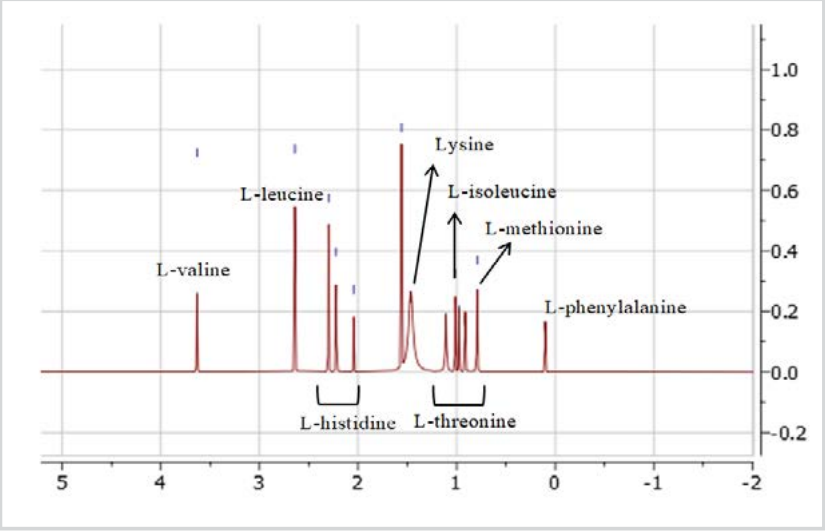

Figure 11H-NMR spectrum of Paralysis group's cerebrospinal fluid. Detected chemical shifts (ppm) for L-phenylalanine (0.06-0.25), L-isoleucine (0.73-2.46, methine group), L-histidine (0.78-2.34, imidazole ring), Lysine (0.73-2.19), L-valine (1.46-3.65), L-tryptophan (0.13-0.65, amine group), L-threonine (0.59-2.38, methine group), L-leucine (0.66-2.64), L-methionine (0.29-1.46).

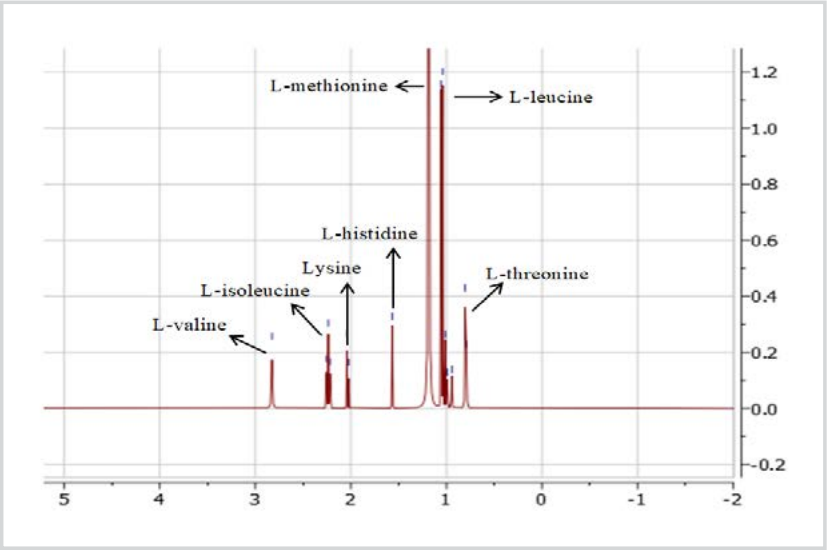

Figure 2 1H-NMR spectrum of Healthy group's cerebrospinal fluid. Detected chemical shifts (ppm) for L-phenylalanine (0.06-0.25), L-isoleucine (0.73-2.46, methine group), L-histidine (0.78-2.34, imidazole ring), Lysine (0.73-2.19), L-valine (1.46-3.65), L-tryptophan (0.13-0.65, amine group), L-threonine (0.59-2.38, methine group), L-leucine (0.66-2.64), L-methionine (0.29-1.46). L-phenylalanine cannot be visualized due to its low concentrations.

DISCUSSION AND CONCLUSION

Tick paralysis is caused by neurotoxins secreted by adult female ticks, primarily *Ixodes holocyclus*. However, there are some reports of *R. sanguineus* being a cause of paralysis in dogs (Otranto et al., 2012). These toxins inhibit the presynaptic release of acetylcholine into the synaptic space of the neuromuscular junction, leading to various clinical manifestations, ranging from rare isolated cranial nerve involvement to severe quadriplegia and paralysis of the respiratory muscles. In critical cases, paralysis of the respiratory muscles without access to mechanical ventilation can result in death. Therefore, early diagnosis of tick paralysis is vital due to its highly variable prognosis (Gülersoy et al., 2024c). While certain omics approaches have been explored in the context of tick vaccination and potential diagnostic or prognostic metabolomics in affected dogs, research on 1H-NMR-based CSF evaluation in cases of tick paralysis has been notably absent (de la Fuente and Merino, 2013). This study, in which potential diagnostic and/or prognostic markers were investigated in CSF samples of dogs affected by tick paralysis using a 1H-NMR based approach, has yielded important results. Tick-paralyzed dogs were observed to have higher concentrations of L-phenylalanine, L-isoleucine, L-histidine, lysine, and L-tryptophan, likely as a neuroprotective response to neuroinflammation, and lower concentrations of L-threonine, L-leucine, L-methionine, and L-valine, attributed to an impaired transfer mechanism and ongoing neuronal damage. The amino acids with altered expression detected in this study, along with this approach, may provide insights into the pathological mechanisms underlying tick paralysis. Additionally, these metabolites with altered expression could serve as potential diagnostic and prognostic markers, as

the prognosis of tick paralysis is highly variable, especially when diagnosis is delayed, particularly in atypical cases where ticks cannot be found on the host. Furthermore, the findings may also contribute to research on other non-infectious acute flaccid paralysis conditions.

If left untreated, tick paralysis can progress to respiratory failure and potentially result in death. Therefore, healthcare workers must be well-acquainted with this relatively rare but treatable cause of acute motor weakness, maintaining a high level of suspicion to avoid delays in diagnosis and treatment (Padula et al., 2020). Unlike assessments that focus solely on gait and respiratory scores, the MGCS evaluates the overall neurological status, making it a more comprehensive tool for monitoring patient condition (O'Keeffe and Donaldson, 2023). Thus, the MGCS was chosen for its ability to provide vital prognostic assessment for both veterinarians and owners, allowing for the grading of initial neurological status, including gait, respiratory pattern, and serial patient follow-up (Platt et al., 2001). Clinical examinations of dogs with tick paralysis often reveal fever, altered mental status, and increased respiratory rate, all of which were evaluated using the MGCS in the present study. The cardiovascular system is also adversely affected, leading to an increased heart rate, elevated blood pressure, irregular heart rhythms, and coagulopathies (Shaffran, 2008). The elevated body temperature, pulse, respiratory rate, and shortened CRT observed in the tick-paralyzed dogs in the present study align with previous findings and may be attributed to a hypermetabolic reaction of skeletal muscles to the tick neurotoxin (Padula, 2016).

Little is known about the pharmacological mechanism of tick toxin action (Simpson, 1996; Padula, 2016). The toxin binds with high affinity to receptors on nerve endings, penetrates the cell membrane via receptor-mediated endocytosis, and crosses the endosomal membrane through pH-dependent translocation. Once in the cytosol, the toxin acts as a zinc-dependent endoprotease, cleaving polypeptides essential for exocytosis. The absence of these polypeptides prevents nerve action potentials from triggering acetylcholine release, a process thought to be enzymatically mediated (Simpson, 1986; Padula 2016; Simon et al., 2023). Acute flaccid paralysis (AFP) typically reaches maximum severity within days to weeks, with the progression timeline depending on the specific etiology (Bowley and Chad, 2019). In the brain, supraphysiological glutamate release induces neurotoxicity. Therefore, the search for effective neuroprotective agents has centered on compounds that block glutamate receptors or inhibit glutamate release. Drugs capable of targeting multiple excitotoxic pathways may offer superior neuroprotective efficacy. Previous studies suggest that the aromatic amino acid L-phenylalanine, an endogenous substance, may exhibit such properties (Kagiyama et al., 2004). Although tick paralysis primarily affects peripheral neurons, brainstem dysfunction and autonomic dysregulation can also occur (Hegen et al., 2021; Gülersoy et al., 2024c). The elevated CSF L-phenylalanine concentrations observed in this study may represent a neuroprotective response by the body. Therefore, L-phenylalanine supplementation, along with monitoring its concentrations in both serum and CSF, may serve therapeutic and prognostic purposes. However, it should be kept in mind that a key challenge for neuroprotective compounds is achieving concentrations sufficient for neuroprotection without causing adverse effects on the cardiovascular or nervous systems.

Histidine is an essential amino acid in dogs, playing critical roles in various metabolic processes, including its involvement in the histaminergic system of the central nervous system. It also serves as a precursor to histamine, a molecule that contributes to inflammatory processes and the pathogenesis of multiple sclerosis (Shmalberg, 2015; Židó et al., 2023). Elevated L-histidine concentrations in cerebrospinal fluid (CSF) have also been reported in individuals with Alzheimer's disease, although the specific nature of this role remains unclear (Kaiser et al., 2007). Animal models suggest that increased histamine levels may drive the synthesis of pro-inflammatory

cytokines, such as TNF and interferon gamma (Židó et al., 2023). In the present study, higher CSF L-histidine concentrations were observed in tick-paralyzed dogs compared to healthy controls. This increase may reflect the dual roles of microglia, which contribute to both neuroprotection and neurodegeneration (Smith et al., 2012). Additionally, this finding aligns with the elevated CSF L-phenylalanine concentrations observed in this study, suggesting a broader metabolic response to tick paralysis.

Lysine is an essential amino acid known to promote protein synthesis and improve neurological function. Previous studies have shown that lysine enhances cerebral blood flow and supports recovery in patients with ischemic stroke through its neuroprotective and neurotrophic effects (Kondoh et al., 2010). Elevated CSF lysine concentrations have also been linked to mental retardation and motor neuron diseases (Cheng et al., 2020). Additionally, increased nitrogen excretion has been observed in certain neuronal diseases. In the present study, the elevated CSF lysine concentrations in tick-paralyzed dogs may be associated with enhanced nitrogen excretion, microglial polarization, neuroprotection, and the prevention of brain cell death (Kondoh et al., 2010; Cheng et al., 2020).

Isoleucine, leucine, valine, phenylalanine, tyrosine, and lysine compete for transport across the blood-CSF barrier via a common carrier system. In humans, this amino acid transport system differs from the neutral amino acid carrier observed at the blood-brain barrier in rats (Oldendorf and Szabo, 1976). Methionine and tryptophan, however, do not compete with other neutral amino acids for this system. Interestingly, lysine, a basic amino acid, has been associated with the same transport mechanism as the five neutral amino acids. Patients with blood-CSF barrier dysfunction for proteins may exhibit partly normal, increased, or decreased CSF concentration quotients for these amino acids (Kruse et al., 1985). Amino acid transport across the blood-CSF barrier is crucial for neuronal metabolism and neurotransmission. The synthesis of neuronal glutamate from α-ketoglutarate requires an amino group nitrogen donor. Among the branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine-these amino acids can serve as nitrogen donors for vesicular neurotransmitter glutamate synthesis. However, a previous study found that only valine sufficiently supports the increased demand for vesicular glutamate synthesis (Bak et al., 2012). In conditions involving blood-CSF or blood-nerve barrier dysfunction, such as Guillain-Barré syndrome (GBS), which is an acute flaccid paralysis similar to tick paralysis, CSF total protein levels are elevated due to the release of myelin proteins from inflamed spinal nerve roots (Hegen et al., 2021). In the present study, increased L-phenylalanine levels, which may serve a neuroprotective function, alongside elevated L-isoleucine and L-valine concentrations and reduced L-leucine levels, suggest an underlying dysfunction of the blood-nerve barrier or blood-CSF barrier.

Threonine serves as a phosphorylation site for numerous enzymes, playing several vital roles in the body. Additionally, it acts as a precursor to glycine, an inhibitory neurotransmitter, thereby influencing neurotransmitter balance in the brain (Shmalberg, 2015). Studies have shown a significant correlation between threonine consumption and its plasma, brainstem, and cortical levels, which are closely associated with cortical concentrations of threonine and glycine (Kaiser et al., 2007). Furthermore, certain genes are implicated in the transport of amino acids, such as serine and threonine, from astrocytes to neurons. While elevated CSF L-threonine levels are linked to neurodegeneration of meningeal cells, neurons, granular cells, and Purkinje cells, the reduced CSF L-threonine concentrations observed in the tick-paralyzed dogs in this study may result from impaired transport mechanisms (Swanson et al., 2022; Gülersoy et al., 2024c). However, these mechanisms need to be investigated in cases of acute flaccid paralysis.

The principal role of tryptophan is as a constituent of protein synthesis. Because it is found in

the lowest concentrations among amino acids, tryptophan is relatively less available and is considered to play a rate-limiting role during protein synthesis. Tryptophan is also the precursor to two important metabolic pathways: kynurenine synthesis and serotonin synthesis (Dougherty et al., 2008; Richard et al., 2009). The kynurenine pathway is a major route for tryptophan metabolism, producing a range of biologically active molecules with properties including oxidants, antioxidants, immunomodulators, neurotoxins, and neuroprotectants. Disruption of the tryptophan-kynurenine metabolism is strongly associated with neuroinflammation and immune activation. Increased production of pro-inflammatory cytokines activates the kynurenine pathway's regulatory enzyme, indoleamine-2,3-dioxygenase (IDO1), along with related enzymes. This causes dysregulation of the pathway, leading to depletion of tryptophan and an imbalance in the formation of neuroprotective (kynurenic acid) and neurotoxic (quinolinic acid, 3-hydroxykynurenine) metabolites (Richard et al., 2009; Mithaiwala et al., 2021). Given that L-tryptophan can cross the blood-brain barrier (Richard et al., 2009), the elevated concentrations of CSF L-tryptophan observed in the tick-paralyzed dogs of the present study may be due to an increased transfer of tryptophan from the blood to the CSF to provide neuroprotection in response to neuroinflammation.

L-methionine is an essential amino acid and a key component of one-carbon metabolism. It is necessary for the production of S-adenosyl methionine, the primary methyl donor in the body, which is involved in nearly all methylation reactions. These reactions target substrates such as ribonucleic acids, proteins, carbohydrates, phospholipids, and neurotransmitters (Trivedi and Deth, 2012). Methylation is a universal biological process critical for cell proliferation, differentiation, survival, and other cellular functions (Roidland Hacker, 2014). Disruption of methionine metabolism has been linked to various neurological and psychiatric disorders. For instance, altered levels of L-methionine and other one-carbon cycle metabolites have been reported in neurodevelopmental disorders like autism and schizophrenia, as well as age-related neurodegenerative diseases such as Alzheimer's disease and vascular dementia (Zuin et al., 2021). High methionine levels have also been associated with neuronal degeneration and vascular dysfunction (Kalani et al., 2019). The immuno-inflammatory role of methionine has been demonstrated in studies showing that methionine restriction in a mouse model of multiple sclerosis reduces T-cell-mediated inflammation in the brain and spinal cord, delaying disease onset and progression (Agbas and Moskovitz, 2009). In the present study, decreased CSF L-methionine concentrations may reflect a neuroprotective response to tick paralysis. However, most studies have focused on methionine metabolism's impact on T cells, with limited understanding of its effects on innate immune cells, such as microglia in the brain. Further research is needed to elucidate these mechanisms.

Certain tick species can release neurotoxins through their salivary glands during blood feeding, leading to tick paralysis, which is characterized by ascending AFP. In severe cases, respiratory failure and death may occur. Metabolomic profiling of amino acids offers a promising approach for uncovering underlying mechanisms, identifying biomarkers, and informing treatment strategies for autoimmune and neurodegenerative diseases. In this study, 1H-NMR-based amino acid profiling of CSF samples from dogs with tick paralysis revealed notable findings. Tick-paralyzed dogs exhibited higher concentrations of L-phenylalanine, L-isoleucine, L-histidine, lysine, and L-tryptophan, alongside lower concentrations of L-threonine, L-leucine, L-methionine, and L-valine. It was interpreted that the increased levels of amino acids, such as L-phenylalanine, L-isoleucine, L-histidine, lysine, and L-tryptophan reflected a neuroprotective response to neuroinflammation. Conversely, the decreased levels of amino acids, such as L-threonine, L-leucine, L-methionine, and L-valine suggest ongoing neuronal damage and disrupted transfer mechanisms. This approach is concluded to offer valuable insights into the

pathological mechanisms underlying tick paralysis and may also contribute to research on other non-infectious AFP conditions.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORS CONTRIBUTION

Conception: EG, CB; Design: EG, AŞ; Supervision: EG, İG; Materials: EK, İG; Data Collection and/or Processing EG, CB, AŞ; Analysis and/or Interpretation of the Data; EG, CB, AŞ; Literature Review:EK, İG; Writing:EG; Critical Review: EG, CB, AŞ

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KOMPARATIVNO PROFILIRANJE CEREBROSPINALNIH AMINOKISELINA KORIŠTENJEM 1H-NMR KOD KRPELJSKI PARALIZIRANIH I ZDRAVIH PASA

SAŽETAK

Krpeljska paraliza uzrokovana neurotoksinima, koje pojedine vrste krpelja otpuštaju sišući krv, dovodi do ascendentne akutne flaksidne paralize (AFP) koja može imati ozbiljne komplikacije, kao što su respiratorna insuficijencija i smrt. Metabolomičko profiliranje aminokiselina, posebno korištenjem 1H-NMR, predstavlja dragocjenu alatku za razumijevanje mehanizama koji uzrokuju ovo stanje. Naše istraživanje je obuhvatilo 92 psa sa kliničkim znacima iznenadne slabosti, otežanog kretanja i gubitka koordinacije stražnjih nogu koji su indikativni na AFP, a koji su evaluirani u bolnici za životinje Veterinarskog fakulteta Univerziteta Harran. Prema uključno/isključnim kriterijima, 15 pasa je svrstano u grupu paraliziranih, a 10 u grupu zdravih. Od svih pasa su prikupljeni uzorci cerebrospinalne tečnosti, pri čemu je na svim uzorcima provedeno 1H-NMR bazirano profiliranje aminokiselina korištenjem Agilent 400 MHz spektrometra. Grupa paraliziranih je pokazala višu tjelesnu temperaturu, srčanu frekvencu i frekvencu disanja u odnosu na grupu zdravih ($p<0,028$). Paralizirani psi su imali kraće vrijeme kapilarnog punjenja ($p<0.008$), dok su zdravi psi imali veći skor na Modificiranoj Glasgowskoj skali kome (MGCS). Što se tiče koncentracije aminokiselina, grupa paraliziranih je pokazala više koncentracije L-fenilalanina, L-izoleucina, L-histidina, lizina i L-triptofana ($p<0.038$), a niže koncentracije L-treonina, L-leucina, L-metionina i L-valina ($p<0.036$). Ovi rezultati pokazuju kako povišene koncentracije određenih aminokiselina odražavaju neuroprotektivni odgovor na neuroinflamaciju, dok snižene koncentracije ukazuju na neuronalno oštećenje i prekid transfernih mehanizama. U cjelini, ovo istraživanje produbljuje razumijevanje krpeljske paralize i može pružiti uvid u druga neinfektivna stanja s AFP.

Ključne riječi: Akutna flakcidna paraliza, biomarker, krpeljska paraliza, pas