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Evaluation of Serum Amino Acid and Carnitine Profile in Dogs with Transmissible Venereal Tumor

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Abstract: The presented study aimed to reveal the changes in serum amino acid and carnitine profiles in dogs with transmissible venereal tumor (TVT). The study material comprised 40 female dogs ranging in age from 3 to 5 years. The dogs were divided into two groups based on genital organ examinations. Group 1 (n=20) consisted of healthy dogs, while Group 2 (n=20) consisted of TVT-positive dogs. Blood samples were taken from dogs in both study groups, and serum was obtained. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for the determination of carnitine and amino acid profiles. The obtained data were compared using an independent samples t-test. The serum amino acid profiles of Lysine, Aspartic Acid, Tyrosine, Asparagine, Alanine, Arginine, Citrulline, Glutamic Acid, Glycine, Methylglutaryl, Phenylalanine, and Ornithine were found to be lower in the TVT group (P<0.05). The serum carnitine profiles of C0 (Free carnitine), C2 (Acetylcarnitine), C4 (Butyrylcarnitine), C4-DC (Methylmalonylcarnitine), C5 (Isovalerylcarnitine), C5:1 (Tiglylcarnitine), C5-OH (3-Hydroxyisovalerylcarnitine), C5-DC (Glutarylcarnitine), C6 (Hexanoylcarnitine), C6-DC (Adipoylcarnitine), C8 (Octanoylcarnitine), C8:1 (Octenoylcarnitine), C8-DC (Suberoylcarnitine), C10 (Decanoylcarnitine), C10:1 (Decenoylcarnitine), C12 (Dodecanoylcarnitine), C14 (Myristoylcarnitine), C14:1 (Myristoylcarnitine), C14:2 (Tetradecadienoylcarnitine), C18:1 (Oleoylcarnitine), C18:2 (Linoleylcarnitine), and C18:1-OH (Hydroxyoleylcarnitine) were found to be lower in the TVT group (P<0.01). The profiles of C3 (Propionylcarnitine) and C16 (Palmitoylcarnitine) were found to be higher in the TVT group (P<0.01). As a result, it was concluded that the significant changes in amino acid and carnitine values could be used as biomarkers for diagnosing TVT in dogs.

Keywords: *Amino acid, Carnitine, Dog, Transmissible venereal tumor.*

Transmissible Venereal Tümörlü Köpeklerde Serum Amino Asit ve Karnitin Profilinin Değerlendirilmesi

Özet: Sunulan çalışmada transmissible venereal tümörlü (TVT) köpeklerde serum amino asit ve karnitin profilindeki değişimleri ortaya koymak amaçlandı. Çalışma materyalini, 3-5 yaşları arasında değişen 40 adet dişi köpek oluşturdu. Köpekler genital organ muayenelerine göre iki gruba ayrıldı. Grup 1 (n=20) sağlıklı köpeklerden, Grup 2 (n=20) ise TVT pozitif köpeklerden oluştu. Her iki çalışma grubundaki köpeklerden kan örnekleri alınarak serum elde edildi. Karnitin ve amino asit profilinin belirlenmesinde, sıvı kromatografi-kütle spektrometresi (LC-MS/MS) kullanıldı. Elde edilen veriler bağımsız gruplar t-test ile karşılaştırıldı. Serum amino asitlerden Lizin, Aspartik Asit, Tirozin, Asparajin, Alanin, Arginin, Sitrülin, Glutamik Asit, Glisin, Metilglutaril, Fenilalanin ve Ornitin profilleri TVT grubunda daha düşük olduğu belirlendi (P<0.05). Serum karnitinlerden C0 (Serbest karnitin), C2 (Asetilkarnitin), C4 (Bütriylkarnitin), C4-DC (Metilmalonilkarnitin), C5 (İzovalerylkarnitin), C5:1 (Tiglikarnitin), C5-OH (3-Hidroksiizovalerylkarnitin), C5-DC (Glutarylkarnitin), C6 (Heksanoilkarnitin), C6-DC (Adipoilkarnitin), C8 (Oktanoilkarnitin), C8:1 (Oktenoylkarnitin), C8-DC (Suberoilkarnitin), C10 (Dekanoilkarnitin), C10:1 (Dekenoylkarnitin), C12 (Dodekanoilkarnitin), C14 (Miristoilkarnitin), C14:1 (Miristoilkarnitin), C14:2 (Tetradekadienoylkarnitin), C18:1 (Oleoylkarnitin), C18:2 (Linoleylkarnitin) ve C18:1-OH (Hidroksi-oleylkarnitin) profillerinin TVT grubunda daha düşük olduğu belirlendi (P<0.01). C3 (Propiyonilkarnitin) ve C16 (Palmitoilkarnitin) profilleri ise TVT grubunda daha yüksek tespit edildi (P<0.01). Sonuç olarak amino asit ve karnitin değerlerindeki belirgin değişimler bu iki profilin köpeklerde TVT teşhisinde biyobelirteç olarak kullanılabileceği kanısına varıldı.

Anahtar Kelimeler: *Amino asit, Karnitin, Köpek, Transmissible venereal tümör.*

Introduction

Transmissible venereal tumor (TVT), also referred to as sticker tumor, venereal granuloma, or infectious sarcoma, is defined as a contagious and benign reticuloendothelial tumor that primarily affects the external and sometimes internal genital regions of dogs (Tella et al., 2004). The tumor is usually spread through mating (Mukaratirwa and Gruys, 2003). It can also spread to non-genital areas such as the eyes, nose, and oral cavity through social behaviours like sniffing and licking (Abedin, 2020). In TVT, which typically appears cauliflower-like, ranging from crisp to reddish-brown in color, clinical signs such as pain, bleeding, and serosanguinous discharge from the external genital area are observed (Mac-Ewen, 2001). This tumor continues to be a significant problem in countries where mating is not controlled (Das and Das, 2000).

Amino acids have significant functions in numerous metabolic pathways, serving as both substrates and regulators. Assessing the concentrations of free amino acids in bodily fluids and specific tissues offers valuable insights into the biochemical and nutritional status linked with different diseases (Tochikubo and Ando, 2010). Traditionally, tumor metabolism has predominantly centered around carbon metabolism, particularly glycolysis and the tricarboxylic acid cycle. However, recent research has illuminated the significance of amino acids in cancer metabolism. Although glucose is a recognized energy source for tumor growth, amino acids also play a significant role as fuels that support cancer development (Lieu et al., 2020). Amino acids can serve as alternative fuel sources for cells (Green et al., 2016). All mammalian cells employ cellular metabolism to produce essential biomolecules for energy generation and to maintain homeostasis (Hanahan et al., 2011). Amino acids in tumor cells help meet these requirements by assisting in protein synthesis, energy and nucleotide generation, redox balance maintenance, and epigenetic modification (Pavlova et al., 2016).

Carnitine is a derivative of amino acids consisting of various forms, including free carnitine in its endogenous form and short, medium, and long-chain acylcarnitines (Wolf et al., 2013). Carnitine serves two primary functions: facilitating the transportation of long-chain fatty acids into the mitochondrial matrix for β-oxidation, generating cellular energy, and regulating the high intramitochondrial acyl-coenzyme A (CoA)/CoA ratio, thereby reducing the inhibition of many intramitochondrial enzymes involved in glucose and amino acid breakdown (Sandikci et al., 1999). Additionally, it possesses numerous metabolic roles, including stimulating hematopoiesis, blocking collagen-induced platelet aggregation, and averting programmed cell death in immune cells (Wolf et al., 2013). There is evidence showing that carnitine-mediated fatty acid oxidation in the pathogenesis of tumor development may contribute to the production of adenosine triphosphate (ATP), which could play a critical role in tumor progression (Carracedo et al., 2013). One strategy to meet the heightened energy demand of malignant cell proliferation is by engaging in glycolytic activity (Schmidt et al., 2010). Another approach is to acquire energy through fatty acid oxidation (FAO) from nearby adipose tissue, lipoproteins, and phospholipids (Carracedo et al., 2013).

The objective of this study is to explore the correlation between carnitine and amino acid levels in TVT, aiming to contribute novel perspectives to the existing literature and to ascertain its potential utility as a diagnostic biomarker for the disease.

Materials and Methods

Animal Selection: The study was conducted on 40 female mixed-breed dogs brought from Şanlıurfa Metropolitan Municipality Animal Shelter to Harran University Faculty of Veterinary Medicine Animal Hospital for treatment purposes. The study was carried out from February to May. The study utilized animals selected through a random sampling method, all subjected to identical feeding and management conditions. These animals were between 3 to 5 years of age and had an average weight of 26.73±5.12 kg. Dogs diagnosed with pregnancy, systemic disease, or undergoing chemotherapy for TVT treatment were not included in the study based on routine hematological, biochemical, urine analysis, and ultrasonographic examinations. The dogs were segregated into two groups following genital organ examinations. Group 1 (n=20) consisted of healthy dogs, while Group 2 (n=20) consisted of TVT-positive dogs. Those with swelling in the genital area, conformational abnormalities, excessive licking of the region, unusual odor, and evident cauliflower-like masses were considered positive for TVT. A vaginal smear sample was taken for a definitive diagnosis. A vaginal swab was rotated around its own axis within the vaginal mucosa, without contacting the clitoral fossa and external urethral orifice, at a 45-degree angle dorsally from between the labia, to ensure the collection of an adequate number of cells. The swab sample was immediately used to prepare two smear slides on a slide and stained using the Giemsa staining method without delay. The diagnosis of TVT was confirmed by the detection of intracytoplasmic vacuoles and numerous mitotic figures in the samples examined under a light microscope.

Laboratory Analyses: Blood samples were collected from dogs in both experimental groups via the cephalic vein using a 20G sterile syringe and transferred to 10 mL gel vacutainer tubes. Subsequently, the samples underwent centrifugation at 3000 rpm for 15 minutes to obtain serum. The determination of carnitine and amino acid profiles was performed using the LC-MS/MS device (Shimadzu, Japan) employing the method utilized by Tammo et al. (2021).

Statistical Analysis: Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS 26.0). Data normality was assessed analytically (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics for variables that followed a normal distribution were presented as mean ± standard error of the mean (SEM). Group comparisons were made utilizing the Independent Samples

t-test, given the normal distribution of the data. P<0.05 was defined as statistically significant.

Results

The mean serum amino acid and carnitine values for the study groups are given in Tables 1 and 2, respectively. The profiles of serum amino acids including Lysine, Aspartic Acid, Tyrosine, Asparagine, Alanine, Arginine, Citrulline, and Glutamic Acid (P<0.001); Glycine (P<0.01); and Methylglutaryl, Phenylalanine, and Ornithine (P<0.05) were observed to be lower in the TVT group. The profiles of Valine and Methionine were observed to be not significantly different between the TVT and healthy groups (P>0.05). The profiles of serum carnitines including C0, C2, C4, C4-DC, C5, C5:1, C5-OH, C5-DC, C6, C6-DC, C8, C8:1, C8-DC, C10, C10:1, C14, C14:1, C18:1, C18:2, and C18:1-OH (P<0.001); C12 (Dodecanoylcarnitine) and C14:2 (Tetradecadienoyl-carnitine) (P<0.01) were observed to be lower in the TVT group. The profiles of C3 and C16 were observed to be higher in the TVT group (P<0.01). The profiles of C10-DC (Sebacoylcarnitine), C16:1 (Palmitoleylcarnitine), and C18 (Stearoylcarnitine) were observed to be not significantly different between the TVT and healthy groups (P>0.05).

Table 1. The mean serum amino acid values for the study groups.

Amino Acid Profile (µmol/L)	TVT Positive Group $\bar{X} \pm SEM$	Control Group $\bar{X} \pm SEM$	P value
Methy Glutaryl	0.0215±0.002	0.0280±0.002	**0.043**
Valine	356.13±20.58	388.29±4.21	0.253
Lysine	229.73±11.63	363.41±21.32	**0.000**
Methionine	62.40±3.98	67.27±6.52	0.355
Phenylalanine	62.26±3.97	75.53±1.48	**0.024**
Aspartic Acid	0.0580±0.006	0.1250±0.011	**0.000**
Tyrosine	51.39±2.82	83.97±4.66	**0.000**
Asparagine	50.62±3.81	82.46±6.06	**0.000**
Alanine	806.005±94.66	1413.36±57.46	**0.000**
Arginine	399.77±18.53	575.20±22.48	**0.000**
Citrulline	77.02±3.86	131.83±7.62	**0.000**
Glycine	267.24±5.09	375.55±21.33	**0.001**
Ornithine	30.09±1.95	36.46±1.83	**0.017**
Glutamic Acid	364.81±7.32	590.56±33.50	**0.000**

SEM: Standard error of the mean.

Discussion

Metabolomic analysis of carnitine and amino acids provides promising opportunities to elucidate complex metabolic changes associated with tumors and accelerate the identification of novel tumor biomarkers. Metabolomic profiling can aid in the identification of cancer biomarkers and provide clues for early cancer diagnosis. Amino acids and acylcarnitines, which play critical roles in cell physiology as essential metabolites and metabolic modulators, are potential biomarkers for tumor diagnosis (Aboud and Weiss, 2013). The presented study is the first to elucidate the changes in amino acid and acylcarnitine profiles in dogs with TVT disease, providing crucial data contributing to the understanding of these alterations.

Amino acids, the fundamental building blocks of proteins, have crucial functions in mammalian metabolism, cellular growth, genetic expression, and inflammatory reactions. For tumor cells to proliferate, protein (nitrogen supply) and amino acids (support nucleotide biosynthesis) are required. Alterations in amino acid concentrations can markedly impact the tumor microenvironment and immune response, highlighting modified amino acid profiles in individuals with cancer. It has been reported that there are significant differences in plasma amino acid profiles between early and late-stage cancers in both cancerous and healthy individuals (Ward and Thompson, 2012). Kubota et al. (1992) suggested that patients with different types of cancer exhibit a specific amino acid profile characterized by decreased levels of methionine, lysine, glycine, citrulline, aspartate, arginine, alanine, and phenylalanine, and increased levels of tyrosine, valine, and ornithine. To be more precise, Kubota et al. (1992) reported that plasma amino acid levels, especially alanine and arginine, are frequently elevated in breast cancer. Miyagi et al. (2011) demonstrated lower plasma concentrations of glutamine, citrulline, and arginine in patients with early-stage breast cancer compared to healthy individuals. Vissers et al. (2005) found decreased plasma levels of arginine and tryptophan amino acids in patients with breast cancer at different stages. In addition to findings in breast cancer, Lai et al. (2005) reported that plasma amino acids are often suppressed in gastrointestinal cancers, but no noticeable trend was observed in other types of cancer. Miyagi et al. (2011) also indicated decreased levels of amino acids in patients with colorectal and gastric cancer. Turkoglu et al. (2016) demonstrated increased plasma concentrations of glutamine and glycine, and decreased concentrations of phenylalanine and tryptophan in human ovarian cancer. Alanine and valine have increased in some cases and decreased in others (Turkoglu et al., 2016). In patients with endometrial cancer, plasma levels of phenylalanine, tryptophan, and valine decreased in accordance with age (Ihata et al., 2014). The amino acid profile in different tumor types is believed to stem from

Table 2. The mean serum carnitine values for the study groups.

Carnitine Profile	TVT Positive Group	Control Group	P value
	$\bar{X} \pm SEM$	$\bar{X} \pm SEM$	
C0 (Free carnitine)	84.13±1.38	98.54± 0.41	**0.000**
C2 (Acetylcarnitine)	8.01±0.19	9.71±0.13	**0.000**
C3 (Propionylcarnitine)	1.00±0.12	0.77±0.24	**0.000**
C4 (Butyrylcarnitine)	0.150±0.039	0.159±0.002	**0.000**
C4DC (Methylmalonylcarnitine)	0.031±0.0005	0.044±0.0007	**0.000**
C5 (Isovalerylcarnitine)	0.445±0.011	0.568±0.006	**0.000**
C5:1 (Tiglylcarnitine)	0.079±0.001	0.112±0.002	**0.000**
C5-OH (3-Hydroxyisovalerylcarnitine)	0.104±0.003	0.126±0.002	**0.000**
C5-DC (Glutarylcarnitine)	0.0513±0.002	0.0727±0.001	**0.000**
C6 (Hexanoylcarnitine)	0.0264±0.001	0.0564± 0.001	**0.000**
C6-DC (Adipoylcarnitine)	0.0366±0.001	0.0602±0.001	**0.000**
C8 (Octanoylcarnitine)	0.0524±0.004	0.0859±0.001	**0.000**
C8:1 (Octenoylcarnitine)	0.0246±0.001	0.0497±0.001	**0.000**
C8-DC (Suberoylcarnitine)	0.0315±0.002	0.0796±0.001	**0.000**
C10 (Decanoylcarnitine)	0.2014±0.011	0.3242±0.002	**0.000**
C10:1 (Decenoylcarnitine)	0.231± 0.003	0.2715±0.011	**0.000**
C10-DC (Sebacoylcarnitine)	0.0342±0.001	0.0377±0.002	0.134
C12 (Dodecanoylcarnitine)	0.0917±0.018	0.1447±0.000	**0.001**
C14 (Myristoylcarnitine)	0.1327±0.05	0.1960±0.000	**0.000**
C14:1 (Myristoleylcarnitine)	0.1197±0.004	0.2364±0.000	**0.000**
C14:2 (Tetradecadienoylcarnitine)	0.1000±0.004	0.1241±0.002	**0.001**
C16 (Palmitoylcarnitine)	0.3901±0.010	0.3404±0.003	**0.001**
C16:1 (Palmitoleylcarnitine)	0.0983±0.010	0.0910±0.002	0.289
C18 (Stearoylcarnitine)	0.3876±0.012	0.3723±0.002	1.000
C18:1 (Oleylcarnitine)	0.2509±0.012	0.4120±0.004	**0.000**
C18:2 (Linoleylcarnitine)	0.0624±0.002	0.1234±0.002	**0.000**
C18:1-OH (Hydroxyoleylcarnitine)	0.0178±0.001	0.0328±0.000	**0.000**

SEM: Standard error of the mean.

factors such as the tumor type, size, metabolism, and its impact on organ function where it resides. In a few studies conducted in veterinary medicine, it has been demonstrated that tyrosine levels decrease in dogs with melanoma, while phenylalanine and glutamic acid levels increase in dogs with lymphoma. Additionally, decreased levels of methionine, asparagine, glycine, and alanine have been reported in dogs with lymphoma (Kawabe et al., 2015). In dogs with mammary tumors, it has been reported that levels of methionine, asparagine, alanine, and citrulline decrease (Azuma et al., 2012). In the study presented, similar to other different tumors in the literature, it was observed that the serum amino acid levels significantly decreased in TVT-positive dogs. The reason for this is that rapidly dividing tumor cells utilize basic biosynthetic components (such as lipids, amino acids, nucleic acids, etc.) through aerobic glycolysis to meet their energy demands and obtain additional ATP (Hans et al., 2009). Additionally, disease, infection, and anticancer treatments can alter the utilization, digestion, absorption or of amino acids, leading to increased endogenous protein breakdown and thus changes in amino acid profiles. In cancer, this situation can be more profound due to the metabolic demands of the tumor (Jonker et al., 2012).

Acylcarnitines, which are derivatives of carnitine with acyl groups attached, have a significant function in transporting fatty acids for β-oxidation in both mitochondrial and peroxisomal compartments. Changes in their levels provide insights into disruptions in fatty acid oxidation, amino acid metabolism, and glycolysis, indicating the advancement and progression of cancer (Houten et al., 2020; McCann et al., 2021). Acylcarnitine levels are recognized as important biomarkers in identifying metabolic changes caused by various diseases, including congenital metabolic disorders, depression, metabolic disorders, Alzheimer's disease, diabetes, cardiovascular diseases, and certain types of cancer (Dambrova et al., 2022). Assessing acylcarnitine levels opens up new possibilities for diagnosing and prognosticating cancer. It has been reported that in patients with colorectal tumors, tumor cells accumulate long-chain acylcarnitines differently from normal tissues (Shen et al., 2021). In patients with esophageal squamous cell carcinoma, medium-chain acylcarnitine levels were found to be lower compared to the healthy control group (Xu et al., 2013). In solid tumors, serum levels of medium-chain acylcarnitines are generally low, while serum levels of long-chain acylcarnitines are generally high. There are a few studies reporting the use of acylcarnitines as diagnostic biomarkers

for certain cancers in humans (Lu et al., 2016; Niziol et al., 2018; Zhao et al., 2021). In advanced lung and breast cancer patients, there have been reports of decreased serum acylcarnitine concentrations and increased renal clearance of acylcarnitines (Sachan and Datson, 1987). Significant alterations in levels of short, medium, and long-chain acylcarnitines have been documented in patients diagnosed with hepatocellular carcinoma (Lu et al., 2016). Similar results have been reported in the profiles of acylcarnitines profiles of gliomas (Bogusiewicz et al., 2021). In the diagnosis of breast cancer, C12, C14, and C14:2 acylcarnitines have been recognized as potential biomarkers (Kozar et al., 2021), whereas a correlation has been observed between plasma levels of C2 acylcarnitine and the risk of breast cancer (His et al., 2019). In gastric cancer, the increase of C6DC, C16OH, C6, and C0 acylcarnitines has been reported as potential diagnostic biomarkers (Li et al., 2022). Moreover, it has been found that levels of C3, C4, C5, C14, and C16 acylcarnitines are significantly increased in patients with lung cancer (Ni et al., 2016). The increase in some acylcarnitine levels and the decrease in others observed in TVT-positive dogs in the presented study are consistent with changes in acylcarnitine levels occurring in different tumors, as reported in the literature. These changes are thought to be due to increased metabolic activity associated with TVT. Given that serum carnitine profiles are recognized to change with metabolic status, higher acylcarnitine clearance and excretion of non-acidic acylcarnitine in cancer patients may reflect an increase in metabolic state (Dodson, 1989). Decreased acylcarnitine concentrations may be attributed to increased lipid utilization, decreased production, and increased excretion of acidic acylcarnitines, or a combination of these factors (Dodson, 1989; Sachan and Datson, 1987).

Conclusion

In conclusion, amino acid and carnitine profiles have shown significant changes in dogs with TVT. The significant decreases in amino acid values and the significant decreases and increases in carnitine profiles have led to the notion that these two parameters could be utilized as biomarkers for TVT diagnosis. In further studies, utilizing a larger number of animals and specifically determining which parameters to measure, it has been inferred that a clearer diagnosis of the disease can be achieved.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (21.12.2023, 2023/008 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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