Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 4, 596–600
ISSN 1311-1477; DOI: 10.15547/bjvm.2264

Short communication

BLOOD AND URINE CONCENTRATIONS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN DOGS WITH TUMOURS

TS. T. HRISTOV & R. G. BINEV

Department of Internal Non-infections Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

Hristov, Ts. T. & R. G. Binev, 2021. Blood and urine concentrations of vascular endothelial growth factor in dogs with tumours. *Bulg. J. Vet. Med.,* **24**, No 4, 596–600.

Vascular endothelial growth factor (VEGF) is a potent mitogen for vascular endothelial cells. It improves cell survival, stimulates angiogenesis, inhibits cell apoptosis and strongly enhances vascular permeability. In this study, VEGF concentrations were assayed in blood plasma and urine of 22 dogs with neoplasms (lymphosarcoma, splenic haemangiosarcoma and mammary gland carcinoma) and in 7 healthy dogs by means of ELISA. Average blood plasma VEGF in control dogs was 42.13 ± 7.37 pg/mL, while in dogs with lymphoma – 113.35 ± 16.48 pg/mL, in dogs with haemangiosarcoma – 154.85 ± 48.46 pg/mL and in dogs with mammary gland carcinoma – 104.31 ± 12.45 pg/mL. Urine VEGF concentrations in dogs affected with lymphosarcoma were 712.42 ± 233.85 ng/g uCr, in animals with haemangiosarcoma – 223.50 ± 262.33 ng/g uCr and in those with mammary carcinoma: 1053.92 ± 311.63 ng/g uCr. In healthy controls average urine VEGF was 310.11 ± 28.11 ng/g uCr.

Key words: dogs, blood plasma, tumours, urine, vascular endothelial growth factor

The vascular endothelial growth factor (VEGF) is a glycoprotein with key role in physiological and pathological angiogenesis. It suppresses several mechanisms of apoptosis and thus promotes endothelial cell survival (Ferrara, 2004). VEGF has a potent beneficial effect on vascular permeability and transvascular molecular transport (Takano *et al.*, 2011). Evidence about its role in genesis of various types of tumours was provided (Stefanou *et al.*, 2004; Ghasemi *et al.*, 2012; Zhao *et al.*, 2012). Increased VEGF expression in cancer cells initiates tumour neovascularisation (Bergers & Benjamin, 2003). Substantial increase in blood VEGF levels was reported in mammary gland tumours (Kato *et al.*, 2007), hepatocellular carcinoma (Poon *et al.*, 2003), oral melanoma (Taylor *et al.*, 2007), canine lymphosarcoma (Gentilini *et al.*, 2005), skin malignancies (Sobczyńska-Rak, 2009). Apart in blood, high VEGF concentrations were reported in urine of human patients with prostate gland tumours (Bok *et al.*, 2001) and renal carcinoma (Chang *et al.*, 2001)

as well as in urinary bladder carcinoma in dogs (Mohammed *et al.*, 2002).

The lack of comparative studies on blood plasma and urine VEGF concentrations in dogs with oncological diseases that do not affect the urinary system, was the incentive for this study.

The investigation was carried out with twenty nine dogs referred to the Small Animal Clinic of the Faculty of Veterinary Medicine, Trakia University – Stara Zagora, Bulgaria. They were allotted into four groups:

- Control group of clinically healthy dogs (n=7);
- Group I: dogs with lymphosarcoma (n=6);
- Group II: dogs with splenic haemangiosarcoma (n=8);
- Group III: dogs with mammary gland carcinoma (n=8).

In all dogs diagnosed with tumours, the diagnoses was histopathologically confirmed.

Blood samples were collected from all animals by venepuncture of *v. cephalica antebrachii* in commercial heparinised tubes. For assay of plasma VEGF concentrations, Quantikine Canine VEGF – ELISA (R&D Systems Inc, Catalog Number CAVE00) was used. Blood plasma was obtained by centrifugation of freshly collected heparinised blood samples (5000 rpm) for 30 min. Plasma was harvested immediately after centrifugation and stored at –20 ^{o}C until analysis.

Urine VEGF was assayed in first morning urine after centrifugation (1000 rpm, 20 min) and freezing of the supernatant at –20 ^{o}C within 4 hours from collection as described by Hayward *et al.* (2008). Urine VEGF levels were quantitated by means of Quantikine Canine VEGF – ELISA тест (R&D Systems, Inc, Catalog Number CAVE00). Creatinine in urine samples were determined on automated biochemical analyser Mindray BS-120 – China. Due to differences in the rate of diuresis, urine VEGF concentrations were normalised vs urine creatinine levels and presented as ng VEGF/g urinary creatinine (Reid *et al.*, 2012).

Data were statistically processed with Statistica v. 6.1 software (StatSoft Inc., 2002). All data were expressed as means and SEM and submitted to one-way ANOVA. P values <0.05 were assumed to be statistically significant. Pearson's correlation coefficients were calculated by Minitab 18 software (Minitab Company, Pennsylvania, USA).

Table 1 presents blood plasma VEGF (pg/mL), and urine VEGF in absolute values (pg/mL) and standardised vs urinary creatinine (ng/g uCr). The latter varied within a relatively narrow range: from 196.67 to 414.58 ng/g uCr, 310.11±28.11 ng/g uCr on the average. In urine of dogs with tumours, VEGF (ng/g uCr) was from 2 to 4 times higher compared to healthy dogs. In Group I, average VEGF was 712.42±233.85 ng/g uCr insignificantly different from controls. The highest value in this group was 1596.23 ng/g uCr. In dogs with haemangiosarcoma (Group II), the parameter values were the highest with mean concentration of 1223.5±262.33 ng/g uCr (P<0.01 vs healthy dogs). The highest individual urine VEGF was in this group – 2656.47 ng/g uCr. In five (62.5%) from all 8 dogs, urinary VEGF levels were >1100 (ng/g uCr). Similar tendency was observed in group III, with mean urine VEGF concentration of 1053.92±311.63 ng/g uCr (P<0.05) and highest value in the group of 2285.4 ng/g uCr.

In the three groups of dogs with tumours, strong positive statistically significant correlation was observed between absolute values of urine VEGF and va-

Table 1. Concentrations of vascular endothelial growth factor (VEGF) in blood plasma and urine (absolute values and values standardised vs urinary creatinine) in healthy dogs and dogs affected with lymphosarcoma (Group I), haemangiosarcoma (Group II) and mammary gland carcinoma (Group III). Data are presented as mean ± SEM

	Healthy controls (n=7)	Group I (n=6)	Group II (n=8)	Group III (n=8)
Blood plasma VEGF (pg/mL)	42.13 ±7.37	113.35±16.48	154.85±48.46	104.31±12.45
Urine VEGF – (pg/mL)	175.44±19.87***	501.29±220.96	1125.83±326.67*	885.31±296.83*
Urine VEGF (ng/g uCr)	$310.11\pm28.11^{+++}$	$712.42\pm233.85^{+}$	$1223.50\pm262.33^{++}$	$1053.92\pm311.63^{++}$

*P<0.05; **P<0.01; ***P<0.001 vs blood plasma VEGF concentrations; $^{+}$P<0.05, $^{++}$P<0.01, $^{+++}$P<0.001 vs blood plasma concentrations.

lues, standardised vs urinary creatinine: r=0.922 (P=0.003) for Group I, r=0.772 (P=0.025) for Group II and r=0.946 (P=0.000) for Group III.

The suggestions for high urinary VEGF levels in tumours of kidneys, urinary bladder or prostate gland are confirmed in human (Chang *et al.*, 2001; Sankhwar *et al.*, 2015) and canine research studies (Mohammed *et al.*, 2002). Various reports indicated that the high VEGF urine concentrations were associated with advanced neoplastic growth in renal carcinoma (Chang *et al.*, 2001) and short survival time in prostate gland tumours (Bok *et al.*, 2001). The studies on urinary VEGF excretion in other tumour types are few, and none were found out in dogs.

In the three groups of dogs with malignancies, urine VEGF was elevated. In the lymphosarcoma group the concentrations were insignificantly different than controls. In dogs with haemangiosarcoma, they were the highest (P<0.01 vs controls) and intermediate in dogs with mammary gland tumours (P<0.05 compared to healthy dogs). Studies in men with brain and haematological neoplasms demonstrated that the measurement of VEGF in urine could be a marker for presence of malignancy or a post treatment marker (Chan *et al.*, 2004; Smith *et al.*, 2008). Our results confirmed that not only urinary tract tumours lead to high urinary VEGF concentrations.

Individual urine VEGF levels in all groups with neoplasms were several times higher than respective blood plasma concentrations. This is observed both for absolute (pg/mL), and normalised urine VEGF levels (ng/g uCr). Our data showed that urine VEGF reflected reliably blood concentrations and could be of diagnostic value in different canine malignancies. Also, our study established an exceptionally strong correlation between absolute urine VEGF concentrations and values, normalised vs urinary creatinine. This practically suggest the possibility for direct interpretation of absolute urine VEGF values instead of calculating values standardised with respect to urine creatinine. Further studies will confirm or reject this option.

In conclusion, vascular endothelial growth factor was detected in high concentrations in blood and urine of dogs

with lymphosarcoma, spleen haemangiosarcoma and mammary gland carcinoma. Its assay in urine could be an easy non-invasive method of diagnosis of malignancies in dogs. The possibility for direct interpretation of urine VEGF levels (pg/mL), instead of values normalised vs urinary creatinine (ng/g uCr) would speed-up and facilitate the diagnostic algorithm.

REFERENCES

Bergers, G. & L. E. Benjamin, 2003. Tumorigenesis and the angiogenic switch. *Cancer*, **3**, 401–410.

Bok, R. A., S. Halabi, D. Fei, C. R. Rodriquez, D. F. Hayes, N. J. Vogelzang, P. Kantoff, M. A. Shuman & E. J. Small, 2001. Vascular endothelial growth factor and basic fibroblast growth factor urine levels as predictors of outcome in hormone-refractory prostate cancer patients. A Cancer and Leukemia Group B study. *Cancer Research*, **61**, 2533–2536.

Chan, L. W., M. A. Moses, E. Goley, M. Sproull, T. Muanza, C. N. Coleman, W. D. Figg, P. S. Albert, C. Ménard & K. Camphausen, 2004. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: A longitudinal study of protein kinetics throughout tumor progression and therapy. *Journal of Clinical Oncology,* **22**, 499–506.

Chang, S. G., S.-H. Jeon, S.-J. Lee, J.-M. Choi & Y.-W. Kim, 2001. Clinical significance of urinary vascular endothelial growth factor and microvessel density in patients with renal cell carcinoma. *Urology*, **58**, 904–908.

Ferrara, N., 2004. Vascular endothelial growth factor: Basic science and clinical progress. *Endocrine Reviews*, **25**, 581–611.

Gentilini, F., C. Calzolari, M. E. Turba, C. Agnoli, D. Fava, M. Forni & P. F. Bergamini, 2005. Prognostic value of serum vascular endothelial growth factor (VEGF) and plasma activity of matrix metalloproteinase (MMP) 2 and 9 in lymphoma-affected dogs. *Leukemia Research*, **29**, 1263–1269.

Ghasemi, M., O. Emadian, F. Naghshvar, A. Bekhradnia, S. Abediankenari, L. Larijani & R. Moghimpour, 2011. Immunohistochemical expression of vascular endothelial growth factor and its correlation with tumor grade in breast ductal carcinoma. *Acta Medica Iranica*, **49**, 776–779.

Hayward, R. M., M. J. Kirk, M. Sproull, T. Scott, S. Smith, T. Cooley-Zgela, N. S. Crouse, D. E. Citrin & K. Camphausen, 2008. Post-collection, pre-measurement variables affecting VEGF levels in urine biospecimens. *Journal of Cellular and Molecular Medicine*, **12**, 343–350.

Kato, Y,, K. Asano, T. Mogi, K. Kutara, K. Teshima, K. Edamura, S. Tsumagari, A. Hasegawa & S. Tanaka, 2007. Clinical significance of circulating vascular endothelial growth factor in dogs with mammary gland tumors. *Journal of Veterinary Medical Science*, **69**, 77–80.

Mohammed, S. I., P. Bennett, B. A. Craig, N. W. Glickman, A. J. Mutsaers, P. W. Snyder, W. R. Widmer, A. E. DeGortari, P. L. Bonney & D. W. Knapp, 2002. Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer. *Cancer Research*, **62**, 356–358.

Poon, R. T., C. P.-Y. Lau, S.-T. Cheung, W.-C. Yu & S.-T. Fan, 2003. Quantitative correlation of serum levels and tumor expression of vascular endothelial growth factor in patients with hepatocellular carcinoma. *Cancer Research*, **63**, 3121–3126.

Reid, C. N., M. Stevenson, F. Abogunrin, M. W. Ruddock, F. Emmert-Streib, J. V. Lamont & K. E. Williamson, 2012. Standardization of diagnostic biomarker concentrations in urine: The hematuria caveat. *PLoS One*, **7**, e53354. doi: 10.1371/journal.pone.0053354.

Sankhwar, M., S. Sankhwar, A. Abhishek & S. Rajender, 2015. Clinical significance of the VEGF level in urinary bladder carcinoma. *Cancer Biomarkers*, **15**, 349–355.

Smith, E. R., D. Zurakowski, A. Saad, R. Scott & M. A. Moses, 2008. Urinary biomarkers predict brain tumor presence and response to therapy. *Clinical Cancer Research*, **14**, 2378–2386.

Sobczyńska-Rak, A., 2009. Correlation between plasma VEGF and angiogenesis of skin and subcutaneous tissue cancer in dogs. *Bulletin of the Veterinary Institute in Puławy,* **53**, 503–508.

Stefanou, D., A. Batistatou, S. Kamina, E. Arkoumani, D. J. Papachristou & N. J. Agnantis, 2004. Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in benign prostatic hyperplasia and prostate cancer. *In Vivo*, **18**, 155–160.

Takano, T., T. Ohyama, A. Kokumoto, R. Satoh & Ts. Hohdatsu, 2011. Vascular endothelial growth factor (VEGF), produced by feline infectious peritonitis (FIP) virus-infected monocytes and macrophages, induces vascular permeability and effusion in cats with FIP. *Virus Research*, **158**, 161–168.

Taylor, K. H., A. N. Smith, M. Higginbotham, D. D. Schwartz, D. M. Carpenter & E. M. Whitley, 2007. Expression of vascular endothelial growth factor in canine oral malignant melanoma. *Veterinary and Comparative Oncology*, **5**, 208–218.

Zhao, X., X. Sun & X. Li, 2012. Expression and clinical significance of STAT3, P-STAT3, and VEGF-C in small cell lung cancer. *Asian Pacific Journal of Cancer Prevention*, **13**, 2873–2877.

Paper received 17.06.2019; accepted for publication 27.09.2019

Correspondence:

Ts. T. Hristov,
Department of Internal Non-infections Diseases, Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria,
tel: 00359 42 699534
e-mail: hristov_vet@abv.bg