

Serum sialic acid fractions in Kangal breed bitches with transmissible venereal tumours

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Summary: The serum sialic acid concentration has been reported to be a potentially useful as tumour markers in many types of tumoural disease. The purpose of this study was to investigate of the levels of serum total sialic acid (TSA) and lipid-bound sialic acid (LSA) in transmissible venereal tumours (TVT) in Kangal Breed (Turkish Anatolian Shepherd Dog) bitches. Blood samples were collected from seven untreated bitches with TVT (test group) and eight healthy bitches (control group). Serum lipid bound sialic acid levels determination was done by the spectrophotometric procedure of Katopodis with resorcinol. Serum total sialic acid levels determination was done by the spectrophotometric procedure of Sydow. The mean TSA and LSA levels in the treated and control groups were 13.46±0.66 mg/dl (11.82-16.94) and 6.13±0.46 mg/dl (4.42-7.58); 9.15±0.59 mg/dl (6.28-11.48) and 2.26±0.24 (1.47-3.63) mg/dl, respectively. Both of serum levels of TSA and LSA in the untreated TVT dogs (n: 7) were significantly (p<0.001) higher than in the healthy dogs (n:8) The data revealed significant elevation in sialic acid levels in TVT and suggested potential utility of these parameters in diagnosis of the TVT. Results of this study suggest that TSA and LSA levels in sera may use in diagnosis serve as a valuable in these tumoural diseases

Key words: Canine transmissible venereal tumour, sialic acid, tumour markers.

Transmissible venereal tümörlü Kangal ırkı dişi köpeklerde serum sialik asit düzeyleri

Özet: Çeşitli tümöral hastalıkların tanısı amacıyla serum sialik asit düzeyinden yararlanılmaktadır. Bu çalışmada Kangal ırkı dişi köpeklerde görülen bulaşıcı venereal tümör olgularında serum TSA ve LSA düzeylerinin araştırılması amaçlanmıştır. Bu amaçla 7 tümörlü hastadan ve 8 sağlıklı köpekten kan örnekleri alındı. Serum lipide bağlı sialik asit düzeyleri Katopodis tarafından bildirilen resorsinol yöntemine göre ölçülürken serum total sialik asit düzeyler Sydow tarafından bildirilen yöntemle spektrofotometrik olarak ölçüldü. Test ve kontrol grubuna ait ortalama TSA ve LSA düzeyleri sırasıyla 13.46±0.66 mg/dl (11.82-16.94) ve 6.13±0.46 mg/dl (4.42-7.58); 9.15±0.59 mg/dl (6.28-11.48) ile 2.26±0.24 (1.47-3.63) mg/dl bulunmuştur. Tümör grubunun (n=7) serum TSA ve LSA düzeyleri kontrol grubuna (n=8) oranla anlamlı ölçüde yüksek bulunmuştur. TVT olgularında sialik asit düzeyinin artış göstermesi tanı amaçlı olarak kullanılabilir. Sonuç olarak, serum TSA ve LSA düzeyleri TVT tanısında klinik muayene bulguları ile birlikte değerlendirilebileceği düşünülmektedir.

Anahtar sözcükler: Köpek bulaşıcı venereal tümör, sialik asit, tümör belirteci.

Introduction

Transmissible venereal tumour (TVT) is most common in tropical and subtropical area containing large populations of free-roaming and sexually active dogs. (4,9). Tumour affects the vagina and external genitalia of the bitch and the penis of the dog. Transmission of the tumour occurs at coitus when infected male dogs. Moreover, autotransmission to the nasal and oral mucosa may occur by licking of the tumour. The lesions are the often friable and multilobulated (11). Although TVT is known benign tumours, metastases and malignancy risk may be very highly in some cases. Expression of the tumour is controlled by the immune system, with more rapid growth and metastasis occurring in immune-suppressed animals and pediatrics. Metastasis of TVT to

the skin, regional lymph nodes, eyes, brain, spleen, liver, lungs, pituitary, nose, tongue, lips, mammary region and thoracic and abdominal viscera has been reported (4,5). Diagnosis is based on the history, clinical findings and cytological examination. Exfoliative cytology proved to be safe and easy method for the diagnosis of TVT. Microscopically the tumour cells have poorly defined and lightly staining cytoplasm; are large and round and oval; and are uniform in size, with occasional large, hyperchromatic nuclei (3).

It is known that the surface of cancer cells differs in many structural and functional characteristics from those of normal cells (2). Neoplastic transformation of a variety of cell types is associated with changes in the composition of membrane glycoproteins (25). Sialic

acids, a group of sugars, have a strong electronegative charge and located in the outer cell membrane glycoconjugates in higher organisms. A small portion of the total sialic acid (TSA) is free in tissues and body fluids; most is bound to glycoprotein and glycolipid (20). They participate of the many biological and pathological processes including metastatic spread and tumor antigenity (16). Increased concentration of sialic acid at the surface of malignant cells in animal and human systems has been related to potential malignancy and changes in immunogenicity (18). Although not much is known about the reasons for the increased sialylation and sialyltransferase activity observed in many cancer cells, often tumour cells seem to be efficiently protected from immune defense by highly sialylated surfaces; this may contribute to the spread of the tumour (16). In many types of neoplasia, serum TSA and LSA levels are diagnostic and prognostic indicator and more suitable for monitoring disease extent and anticancer therapy (1,13,17) Also, Thougard et al. (22) reported that TSA might also be useful as a tumour marker in the dog. The aim of this study was to find out whether there is any correlation between the levels of sialic acid in serum and the canine TVT for using in diagnosis.

Material and Methods

Seven bitches, with naturally occurring TVT admitted to University of Adnan Menderes, Faculty of Veterinary Medicine, Clinic of Obstetrics and Reproductive Diseases, were included in the study group. All of the bitches in this group were Turkish Anatolian Shepherd dogs. The mean age of the bitches was 6.71 ± 2.54 years (1-17 years) and mean body weight was 25.28 ± 1.77 kg (21-33 kg). The diagnosis for the tumour was based on clinical findings and cytological examination. Vaginal smears were stained using Papanicolaou method and examined microscopically. The duration of illness was between 1 and 7 months prior to the chemotherapy. All clinical signs were serosanguineous or bloody vaginal discharge, protrusion of a mass from the rima vulva, licking and odor. In the radiographic and ultrasonographic examination, no metastatic focuses could be detected.

Eight healthy female dogs between the ages of served as the control group. All of them in this group were also Turkish Anatolian Shepherd dogs. The mean age of the bitches was 5.00 ± 1.77 years (1-14 years) and mean body weight was 25.12 ± 2.62 kg (22-30 kg). After bitches in control group were examined generally, it was confirmed that the individuals were not suffering from any inflammatory and metabolic disease. The absence of TVT cells was verified by microscopical examining in Papanicolaou-stained vaginal smears.

Vaginal cytology and tumour stage

For the cytological diagnosis of the tumour, exfoliative cytology was used. Cotton swabs were wetted with saline and inserting into vagina. After fixation in ether-ethanol, smears were stained by using Papanicolaou method. TVT cells were defined as the round to ovoid cells with distinct cytoplasmic borders, round nuclei with one or two prominent nucleoli and moderate amount of faintly light violet cytoplasm in all cytological slides. In addition, the dogs with tumours were staged at clinical according to the World Health Organization staging system (12) after cytologic confirmation. T: Primary tumour size; N: Regional lymph nodes (inguinal) and M: Distant metastasis detected clinical, radiographic and ultrasonographic examination. It was examined their skin, eyes, nose, tongue, lips clinically, genital tract and abdominal organs ultrasonographically and lungs radiographically, respectively.

Sample collection

Serum samples for sialic acid and lipid bound sialic acid levels determination were obtained from venous blood after 12 h fasting by centrifugation of the clotted specimen within 30 min. Sera were separated and stored at -20°C until analyses. The bitches did not receive chemotherapy (vincristine sulfate) before blood samples were collected. Analyzing were performed in Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry.

Biochemical analyses

Estimation of total sialic acid: Serum total sialic acid levels were detected as described by Sydow et al. (21) Briefly, 400 μl of serum were treated with 3 ml of 5% perchloric acid for 5 min at 100°C and centrifuged at 1400 g for 4 min. 2 ml of supernatant were mixed with 400 μl of Ehrlich reagent (5g p-dimethylaminobenzaldehyde/50ml HCl/ 50ml distilled water). After incubation at 100°C for 15 min, 2 ml of distilled water were added on sample and a spectrophotometer (Shimadzu, UV-1601) was used to read the optical density at 525 nm.

Estimation of lipid bound sialic acid: Serum LSA levels were measured as described (6). Briefly, 50 μl of serum was extracted with chloroform-methanol (2:1 v/v) maintained at 4°C . The lipid extract was separated with 0.5 ml of distilled water. The aqueous layer was precipitated with phosphotungstic acid. The precipitates were re-suspended in 1 ml of distilled water and lipid-bound sialic acid in suspension was determined with resorcinol reagent. The TSA and LSA contents were calculated using standard curves obtained for various concentration of N-acetyl neuraminic acid (Sigma, A-0812).

Statistical analysis

The biochemical data are expressed as mean \pm standard error. Student's *t* test was carried out for comparison between the two groups.

Results

The mean serum sialic acid fractions levels in bitches with TVT and controls are shown in Table 1. In TVT group, the mean of serum TSA and LSA concentrations were 13.46 \pm 0.66 mg/dl and 6.13 \pm 0.46 mg/dl respectively. In the control group, the mean of serum TSA and LSA concentrations were 9.15 \pm 0.59 mg/dl and 2.26 \pm 0.24 mg/dl also. Serum TSA and LSA levels were significantly higher in bitches with TVT than healthy bitches (*p* <0.001).

Table1. Mean serum levels of sialic acid fractions in bitches with TVT and in healthy bitches

Tablo 1. Sağlıklı ve tümörlü köpeklerdeki ortalama serum sialik asit düzeyleri

Group	TSA(mg/ dl)	LSA(mg/ dl)
TVT (n=7)	13.46 \pm 0.66	6.13 \pm 0.46
Healthy (n=8)	9.15 \pm 0.59	2.26 \pm 0.24
t	4.81***	7.69***

***: *p* <0.001

The age, clinical signs and individual serum levels of sialic acid fractions in bitches with TVT are summarized in Table 2. The serum TSA concentrations were ranged from 11.82 to 16.94 mg/dl. The levels of LSA were found ranged from 4.42 to 7.58 mg/dl. All clinical signs were serosanguineous or bloody vaginal discharge, protrusion of a mass from the rima vulva, licking and odor. Protrusion of a mass from the rima vulva was observed in three cases.

Serum TSA and LSA concentrations of control group are shown individually in Table 3. Serum TSA and LSA concentrations found ranged from 6.28 to 11.48 mg/dl and 1.47 to 3.63 mg/dl respectively. (Table3).

Table2. Age, body weight, clinical signs and serum levels of sialic acid fractions in bitches with TVT

Tablo 2. Tümörlü köpeklerin yaş, vücut ağırlığı, klinik semptomları ve serum sialik asit düzeyleri

Dog No	Age (year)	Body weight (kg)	Clinical signs	Tumoral stages (TNM)	TSA (mg/ dl)	LSA (mg/ dl)
1	3	21	Bloody vaginal discharge, licking,	T ₂ N ₀ M ₀	12.48	7.58
2	17	33	Protrusion of a mass from the rima vulva, bloody vaginal discharge, licking, odor	T ₃ N ₁ M ₀	11.82	5.89
3	2	22	Bloody vaginal discharge, licking, odor, lower urinary tract infection	T ₂ N ₁ M ₀	13.35	4.42
4	9	26	Serosanguineous vaginal discharge, licking	T ₂ N ₁ M ₀	11.86	7.58
5	10	30	Protrusion of a mass from the rima vulva, bloody vaginal discharge, licking, odor	T ₃ N ₁ M ₀	13.84	6.53
6	5	21	Serosanguineous vaginal discharge, licking	T ₃ N ₁ M ₀	13.93	6.11
7	1	24	Protrusion of a mass from the rima vulva, bloody vaginal discharge, licking, odor	T ₃ N ₂ M ₀	16.94	4.84

Table3. Age, body weight and serum levels of sialic acid fractions in healthy bitches

Tablo 3. Sağlıklı köpeklerin ait yaş, vücut ağırlığı ve serum sialik asit düzeyleri

Dog No	Age (year)	Body weight (kg)	TSA (mg/ dl)	LSA (mg/ dl)
1	3	27	7.97	2.53
2	6	24	6.28	3.63
3	14	22	8.63	2.22
4	3	25	10.90	1.68
5	2	30	9.46	1.47
6	1	23	11.48	2.66
7	6	27	8.47	1.88
8	5	23	10.08	2.08

Discussion and Conclusion

The increasing activity of sialyl transferase is one of the most important changes in sialic acid metabolism of neoplastic cells. Often tumour cells seem to be efficiently protected from immune defense by highly sialylated surfaces; this may contribute to the spread of the tumour (16). Though the exact cause of increase in sialic acid levels in malignancy is not known, various theories attributed are: alterations in the cell surface during transformation of a malignant cell, tumour growth stimulating the liver synthesize glycoproteins, increased glycosylation, shedding from tumour cell surface, or possibly a product of tumour itself or constituent of circulating tumour associated antigen (10). This causes excess amount of sialic acid penetration into the sera. Elevated serum TSA, and also LSA have been used to tumour markers for a number of different types of tumour, and explained by a spontaneous release (shedding) of aberrant sialic acid containing cell surface glycoconjugates (1,17). Measurement of these markers is simple, non-invasive, inexpensive and reproducible methods (35).

In recent years, numerous workers have demonstrated elevated sialic acid contents in human suffering from various types of gynecologic (8,10,13,25) and skin (15,20) malignancies. Also, the markedly

elevated total sialic acid levels observed in dogs with several neoplasm (7,14,18,24). However, very limited information is available on the changes of sialic acid in canine genital tumors. A study on serum sialic acids in TVT of Kangal breed bitches has so far not been reported. Accordance with earlier reports, we observed significantly elevations in both TSA and LSA serum levels of bitches with tumors as compared to healthy controls ($p < 0.001$). The markedly elevated TSA and LSA levels observed in the present study could be associated with high proliferative activity of tumour masses.

In conclusion, our results suggest that the serum levels of TSA and LSA may be used in the diagnosis of bitches with venereal tumors along with clinical and cytological examinations.

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Geliş tarihi: 15.01.2007 / Kabul tarihi: 09.11.2007

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