

## Hematological, serum biochemical results, and treatment approach of an Arabian mare with squamous cell carcinoma of the vulva – case report

[Resultados hematológicos, bioquímicos séricos e abordagem de tratamento de uma égua Árabe com carcinoma escamoso de células da vulva - relato de caso]

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### ABSTRACT

A 20-year-old Arabian mare, was brought to the obstetrics and gynecology clinic with the complaint of an unknown wound in the genital area. Although the mare's general condition was good, examination of the genital area revealed a wide-open wound in the vulva and posterior vagina, characterized by fibrotic growth. The biopsy sample was sent to the pathology laboratory. A diagnosis of squamous cell carcinoma (SCC) was made after histopathological examination. Blood samples for serum biochemistry and hematological tests were collected from the jugular vein. Hematological and serum biochemical findings are generally used to diagnose diseases in animals and humans. Hematological and serum biochemical findings are important with genital SCC. Therefore, the findings of these values are given in this case report.

Keywords: mare, serum hematological results, squamous cell carcinoma

### RESUMO

Uma égua de 20 anos (raça árabe), foi levada à clínica de obstetrícia e ginecologia com a queixa de uma ferida desconhecida na área genital. Embora o estado geral da égua fosse bom, o exame da área genital revelou uma ferida amplamente aberta na vulva e na vagina posterior, caracterizada pelo crescimento fibrótico. A amostra da biópsia foi enviada para o laboratório de patologia. Um diagnóstico de carcinoma espinocelular (CEC) foi feito como resultado do exame histopatológico. Amostras de sangue para bioquímica sérica e testes hematológicos foram coletadas da veia jugular. Os resultados hematológicos e bioquímicos séricos são geralmente usados para diagnosticar doenças em animais e seres humanos. Os achados hematológicos e bioquímicos séricos são importantes com o SCC genital. Portanto, as descobertas destes valores são dadas neste relatório de caso.

Palavras-chave: égua, resultados hematológicos séricos, carcinoma espinocelular

### INTRODUCTION

Squamous cell carcinoma (SCC) is among the most common tumors seen in horses. It is found in the genital and extra-genital organs (Sykora and Brandt, 2017). Squamous cell carcinoma accounts for about 20% of all equine

mucocutaneous tumors. It is found in extra-genital organs by 13%. The most prevalent periorbital tumor location is the eyelid, followed by the third eyelid, cornea, conjunctiva, limbus (corneoscleral junction), and orbit (Gearhart *et al.*, 2007; Taylor and Haldorson, 2013).

Etiologically, the new type of equine papillomavirus, *Equus caballus papillomavirus type 2 (EcPV2)* was found responsible (Scase *et al.*, 2010). Known cofactors in the development of SCCs in horses are repeated trauma, solar radiation, age, and breed (Burney *et al.*, 1992; Valentine, 2006). Although there are different treatment options according to the location, spread, size, and treatment amount of the tumor, surgical intervention, cryotherapy, hyperthermia, radiotherapy, chemotherapy, and photodynamic therapy are among the treatment options (Taylor and Haldorson, 2013).

In this case, we aimed to report the histopathological findings, serum biochemical, and hematological results of SCC in a mare, as well as to discuss possible treatment options and treatment costs.

### CASE REPORT

A 20-year-old Arabian mare was brought to the Clinic of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Firat University, with a proliferative mass that was fleshy, raised with an irregular surface, and multifocal necrotic areas around the vulva and vagina. The mass was 10 x 15 cm, raised, white, firm, and multilobulated (Fig 1). An informed consent form (patient registration protocol no: 203578) was obtained from the patient owner for the applications on the mare.

On clinical examination, the general condition of the mare was good. In the examination of the genital area with inspection and speculum, no lesion was found in the area up to the cervix, and the wound was limited to the posterior part of the vagina and the lips of the vulva. It was observed that the vaginal mass did not damage the anal sphincter. Rectal palpation and transrectal ultrasonography showed normal genital organ morphology. Tissue samples were taken for histopathological examinations after clinical examinations. Tissue samples for histopathological examinations were fixed in 10% formalin solution. Approximately 5µm thick sections were prepared from paraffin blocks prepared by routine methods, stained with the hematoxylin-eosin (H&E) method (Luna, 1968), and examined under a light microscope.



Figure 1. Appearance of tumoral tissue on the lips of the vulva in the mare.

Blood samples from the jugular vein were taken into tubes (BD vacutainer, Plymouth UK) for hematological analysis (containing EDTA) and serum biochemical analysis (without anticoagulant). The serum was separated by centrifugation at 4,000 rpm for 10min. The serum was stored at -18°C till biochemical analysis. Serum biochemical analyses were performed with the automatic biochemistry analyzer device (Gesam Chem 200, Italy).

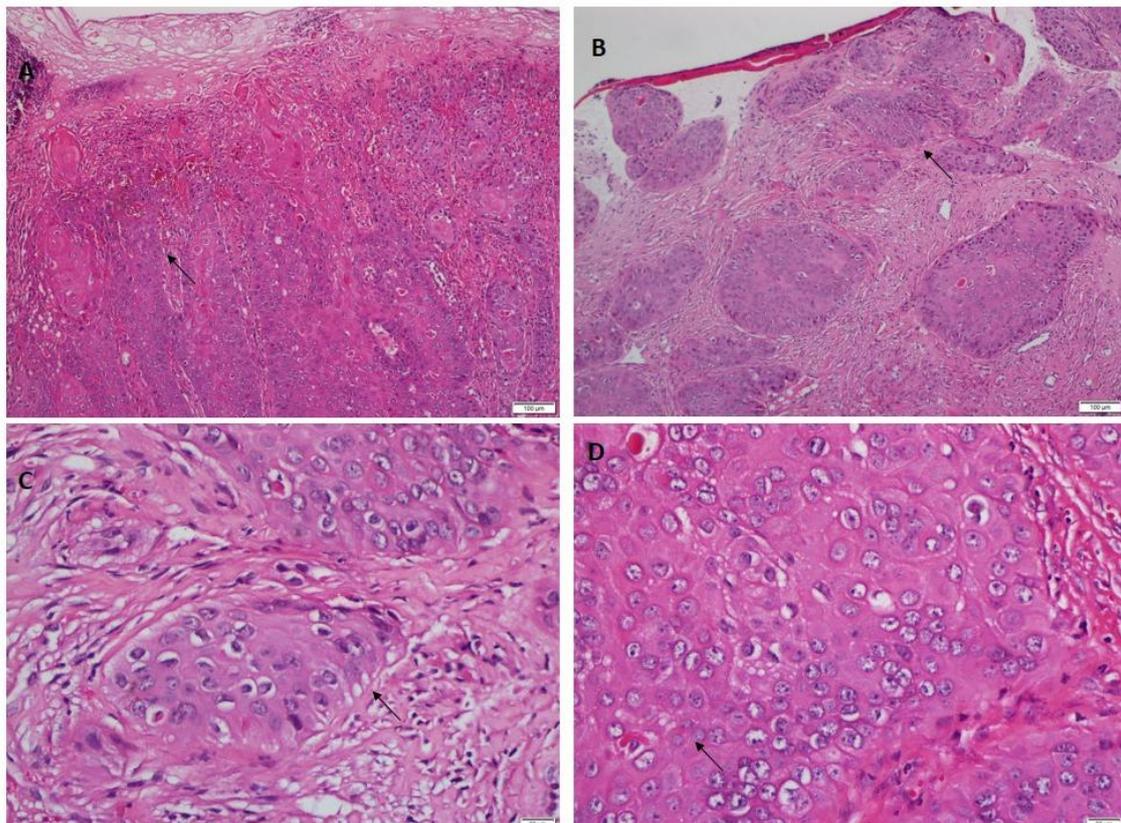
Hematological analyses [white blood cells (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean platelet volume (MPV), red cell distribution width (RDW), platelet (PLT), granulocyte (GRAN), lymphocyte (LYM), and white blood cells not classified as lymphocytes or granulocytes (MID)] were performed using an automatic hematology analyzer device (Mindray bc 5000vet, China).

In the histopathology examination, it was observed that polyhedral cells resembling stratum spinosum cells formed deep nests on the dermal side of the basal cell layer and basal lamina (Fig 2A, 2B). Tumor cells were spreading in the dermis by forming cell cords that anastomose with each other, and some cells remained in the form of isolated islets in the dermal stroma except for these cords (Fig. 2C). Keratinization in such cell cords and islets was found to result in stratified keratin pearls surrounded by tumor cells (Fig 2B). The borders in the cells inside the islets were lost and the cell nuclei were vesicular (Fig 2D). It was noted that most of the tumor cells resemble normal stratum spinosum cells, but the nuclei are vesicular and have one or more prominent nuclei. As a result, the case was anatomically-pathologically diagnosed with SCC.

Hematological results are shown in Table 1. According to the results, while WBC ( $13.7 \times 10^3 \mu\text{l}$ ), MID (46.0%), MCV (80.3 fL), MPV (9.6 fL), and MCH (20.9 pg) increased, RBC ( $5.78 \times 10^6 \mu\text{l}$ ), HCT (26.4%), and MCHC (26.0 g/dL) decreased.

While aspartate aminotransferase (AST) (224.2 IU/L) decreased, urea (46.0 mg/dl) concentrations increased according to serum biochemistry results, other parameters were within normal limits (Table 2).

It has been reported that the recommended chemotherapy and other treatment options will not be accepted by the patient's owner due to the age of the mare and the cost of treatment and will be returned to the former owner.



**Fig 2A:** Polyhedral cells resembling stratum spinosum cells forming extensions towards the lower parts (arrow), H-E, Bar: 100 $\mu\text{m}$ . **B:** Cell groups containing keratin because of loss of intercellular bridges (arrow), H-E, Bar:100 $\mu\text{m}$ . **C:** Groups of tumor cells remaining in the form of isolated islets in the subepithelial stroma (arrow), H-E, Bar:20 $\mu\text{m}$ . **D:** Cells with vesicular nuclei whose boundaries have disappeared in the cells within the islets (arrow), H-E, Bar:20 $\mu\text{m}$ .

Table 1. Hematological results of a mare with squamous cell carcinoma

Parameter (Unit)	Result	Reference range
WBC ( $\times 10^3 \mu\text{l}$ )	<b>13.7</b>	6.0- 12.0
LYM (%)	22.8	20.0- 80.0
MID (%)	<b>46.0</b>	2.0- 8.0
GRAN (%)	31.2	20.0- 70.0
RBC ( $\times 10^6 \mu\text{l}$ )	<b>5.78</b>	6.0- 12.0
HGB (g/dL)	12.1	10.0- 18.0
HCT (%)	<b>26.4</b>	32.0- 48.0
MCV (fL)	<b>80.3</b>	34.0- 58.0
MCH (pg)	<b>20.9</b>	13.0- 19.0
MCHC (g/dL)	<b>26.0</b>	31.0- 37.0
RDW-SD (fL)	44.6	0.1- 99.9
RDW-CV (%)	19.1	15.0- 21.0
PLT ( $\times 10^3 \mu\text{l}$ )	426.0	100- 600
MPV (fL)	<b>9.6</b>	5.0- 9.0

Table 2. Serum biochemical results of a mare with squamous cell carcinoma

Parameter (Unit)	Result	Reference range
ALP (IU/L)	216.0	143.0- 395.0
ALT (IU/L)	6.0	3.0- 25.0
GGT (IU/L)	17.0	7.0- 54.0
AST (IU/L)	<b>224.2</b>	<b>226.0- 366.0</b>
Albumin (g/dl)	2.6	2.6- 3.7
Total bilirubine (mg/dl)	1.03	1.0- 2.0
Creatinine (mg/dl)	1.23	1.2- 1.9
Total protein (g/dl)	8.5	5.2- 7.9
Urea (mg/dl)	<b>46.0</b>	<b>10.0- 24.0</b>
Ca (mg/dl)	11.9	10.2- 13.4
Mg (mg/dl)	1.0	1.4- 2.34
P (mg/dl)	2.58	1.5- 4.7

Ca: calcium, Mg: magnesium, P: phosphorus

## DISCUSSION

Studies are showing the effectiveness of chemoradiotherapy with radiotherapy and a combination of cisplatin and 5-fluorouracil in cases of vulvar SCC observed in humans (Cunningham *et al.*, 1997). In horses, Theon *et al.* (2007) and Fortier and MacHarg (1994) stated that cisplatin and 5-fluorouracil treatment were used in the case of SCC. A case of vulvar SCC infected with papillomavirus developing in a 25-year-old mare has been reported. In another study, laser excision of the tumoral tissue and injection of 5-Fluorouracil around the vulvar tissue was applied as treatment, and the treatment continued until the recurrence of the lesions did not occur (Smith *et al.*, 2009). In this case, the owner refused the treatment protocol because the treatment costs were high. There are different treatment options for the treatment of SCC. However, in field conditions, it is decided

whether the treatment should be performed or not, considering the age of the patient and treatment costs.

The assays included in the biochemical results in SCC will vary in diagnostic procedures and between laboratories. The liver biochemical results may include hepatic enzyme, protein, and total bilirubin assays. However, complete horse results can include all assays completed on a laboratory's automated instrument that have any potential value in horses (Stockham, 1995). The higher than normal serum alkaline phosphatase (ALP) activity recorded in the mare may be the result of possible tumor metastasis to the bone or liver, or specific ALP enzyme induction by neoplastic cells (Kumar *et al.*, 2017). Agina and Ihedioha (2016) high serum ALP activity was noted with high serum total protein level, which is mainly due to high serum globulin concentrations. Also, the serum AST and alanine

aminotransferase (ALT) were slightly lower than normal. In the presented case, ALP, ALT, and  $\gamma$ -glutamyltransferase (GGT) were within normal limits, while AST was below the lower limit. While it was stated that serum creatinine level was high (Agina and Ihedioha 2016), creatinine level was measured within normal limits in the presented case.

Because there are not many studies of this type, the results could not be adequately compared with other studies. Further studies are needed to better understand and characterize the biochemical and hematological results in serum versus an etiological factor of SCC in mare. Based on the results of our studies, while AST concentration decreased and urea concentration increased in mare serum with squamous cell carcinoma, it was also determined that WBC, MID, MCV, MPV, and MCH concentrations increased and RBC, HCT, and MCHC concentrations decreased. Besides, it was concluded that presenting this case to the scientific world would be beneficial for veterinarians, veterinary students, and scientific research.

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