

PRIKAZ SLUČAJA – Case Study

ORAL SQUAMOUS CELL CARCINOMA IN RHESUS MACAQUE (MACACA MULATTA)

PLANOCELULARNI KARCINOM U USNOJ ŠUPLJINI REZUS MAJMUNA (MACACA MULATTA)

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Abstract – This paper describes an oral squamous cell carcinoma with metastases into the lung in a 24-years-old female rhesus macaque (*Macaca mulatta*). At necropsy, 3.5 cm ulcerative, white grey, irregular mass with necrosis and loss of the muscle, and osteolysis of the mandibular bone was observed on the mentum. Histopathologically, the mass consisted of multiple highly cellular lobules of oval to polygonal cells in the abundant connective tissue stroma, and with high mitotic index. There were multifocal pearl-like foci of keratinized cells. Neoplastic cells were PAS negative. Immunohistochemically, positive labeling for high molecular weight cytokeratine (CK_{hwm}) and negative labeling for CA antigen were obtained.

Key words: neoplasia, histopathology, immunohistochemistry, monkey

Kratak sadržaj – U radu se opisuje planocelularni karcinom u oralnoj šupljini s metastazama u plućima ženke rezus majmuna (*Macaca Mulatta*) stare 24 godine. Na obdukciji je u području mentuma ustanovljena ulcerisana, bijelo-siva, nodularna masa nepravilnog oblika, promjera 3,5 cm, s nekrozom mišićnog tkiva i osteolizom simfize mandibule i ispadanjem sjekutića donje vilice. Histopatološki, masa se sastojala od multiplih lobula, jako celularnih, s ovalnim do poligonalnim ćelijama u obilnoj vezivno tkivnoj stromi i velikim mitotičkim indeksom. Uočena su multipla perloidna žarišta keratiniziranih ćelija. Neoplastične ćelije su bile PAS negativne. Imunohistohemijski ustanovljena je jaka pozitivnost ćelija na citokeratin visoke molekularne mase (CK_{hwm}), a negativnost na CA antigen.

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Ključne riječi: neoplazija, histopatologija, imunohistohemija, majmun

Introduction

Squamous cell carcinoma (SCC) is the most commonly described neoplasm in non-human primates such as rhesus macaques (*Macaca mulatta*, squirrel monkeys (*Saimiri sciureus*), common marmosets (*Callithrix jacchus*) and tupai (*Tupaia* spp.) (5, 7). It is one of the most commonly described neoplastic lesions in old monkeys in the zoo gardens. This tumor is of a great importance because of anatomic and physiologic resemblance of non-human primates and humans (3). Also, rhesus macaques are largely used in different fields of biomedical, pharmacological and animal behavior investigations (6). Squamous cell carcinoma represents neoplastic proliferation of cells with squamous differentiation (3). In rhesus macaques, SCC most commonly occurs in the oral cavity, esophagus, vagina and penis (2, 3, 5). Predisposing factors to SCC are exposure to the sunlight and chronic irritation (3, 4).

Case history

A twenty four-year-old female rhesus macaque was kept in a cage with three other adults, two of them were males. The cage was built from glass with metal doors. The temperature in the cage was 23 to 26 °C. Animals were fed fruits, vegetables, cooked eggs, honey and chamomile tea three times a day. Vitamins and minerals were added at need and prescribed by the official veterinarian. The water was at libitum. During the lifespan, no experiments were performed on these animals.

Over time, ulcerative wounds emerged on the mentum of the lower jaw, and were treated with 3% hydrogen peroxide, antibiotic creams and amoxicillin trihydrate i/m. Combination of amoxicillin and clavulanic acid in tablets was administered over ten days. For sedation, tiletamine HCl and zolazepam HCl were used. Following the therapy there was a short recovery, but 35 days later the wounds reemerged and euthanasia was elected because of the poor prognosis.

After death, the animal was presented to necropsy at the Department of Pathology at the Veterinary Faculty, University of Sarajevo. At necropsy, the samples of the mass were collected for histopathology and fixed overnight in 10% phosphate-buffered formalin. The samples were embedded in paraffin blocks, cut into multiple 4-5 microns slices and stained with routine hematoxylin and eosin, and PAS (Periodic Acid Schiff). Sections were also stained immunohistochemically with standard ABC method (Vectastain ABC Universal kit, USA) for pan-cytokeratin (Dako) and carcinoembryonic antigen (CA antigen) (Dako). Antigen unmasking was performed by heat induced epitope retrieval (HIER) using Target Retrieval Solution pH 9 (TRS pH 9; Dako) 3-in-1 as retrieval buffer. Endogenous peroxidase was blocked in 3% H₂O₂ in methanol for 20 minutes. Primary antibody used for pan-cytokeratin was mAb clone cocktail AE1/AE3 (Dako) diluted 1:50. For CA antigen, mAb Clone II-7 (Dako) diluted 1:400 was used.

The reaction was visualised with ABC kit for 30 minutes, followed with diaminobenzidine (DAB) as chromogen for up to 5 minutes. For washing steps and dilutions of antigens phosphate buffered saline (PBS), pH 7,6 was used. Sections were counter-stained with Hematoxylin, processed through series of alcohols and mounted in DPX.

Results and discussion

At necropsy, severe cachexia was present. At the mentum, there was the loss of skin and muscles, and the whole intermandibular region was lost. The oral parts of the mandible (symphysis) as well as teeth were also missing and there was 3.5 cm hard, white, unencapsulated, infiltrative, lobular mass extending into the surrounding skin and muscles. The liver was brown, enlarged, soft and pulpy with multifocal superficial petechial hemorrhages. Multifocal white to grey, nodular foci were noted in the lung parenchyma, and protruding above the surface of the lung.

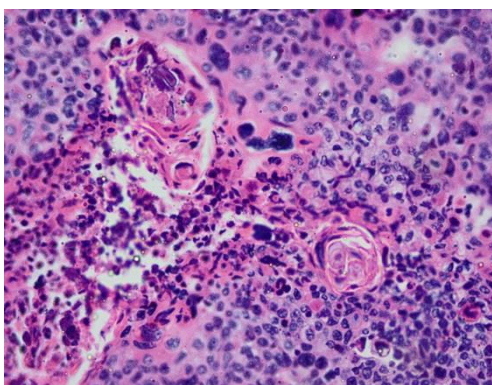


Figure 1.: Oral squamous cell carcinoma; rhesus macaque. Multiple keratinized cells surrounded with inflammatory cells and necrotic debris. Multiple large neoplastic cells with large, irregular, basophilic nuclei are also visible. 200X

Slika 1.: planocelularni karcinom usne šupljine; resus majmun. Multiple keratinizirane ćelije opkoljene upalnim ćelijama i nekrotičnim detritusom. Multiple velike neoplastične ćelije sa velikim, nepravilnim, bazofilnim nukleima su također vidljive. 200X

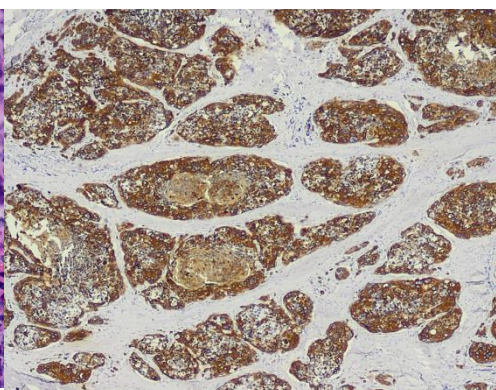


Figure 2.: Oral squamous cell carcinoma; rhesus macaque. Strong cytoplasmic immunoreactivity of the neoplastic cells for pan-cytokeratin. 40X

Slika 2.: planocelularni karcinom usne šupljine; resus majmun. Jaka citoplazmatska imunoreaktivnost neoplastičnih ćelija na pan-citokeratin. 40X

Histology revealed multilobular, unencapsulated, poorly demarcated, infiltrative, and highly cellular mass composed from lobules densely packed with oval to polygonal

cells. The lobules were surrounded with dense bands of fibrous connective tissue. The centers of multiple lobules were filled with eosinophilic, granular necrotic tissue debris, and there were cylindrical keratinized pearls in many of the lobules (Figure 1). There was anisocytosis and anisokaryosis with increased cytoplasm-nucleus ratio and high number of mitotic figures. The neoplastic cells were PAS negative. The immunohistochemistry revealed positive pan-cytokeratin labeling (Figure 2) of the neoplastic cells, but no CA antigen labeling was observed.

Multifocal, multilobular neoplastic foci surrounded with fibrous connective tissue stroma were observed in the lung.

Although squamous cell carcinoma is the most common neoplastic lesion in the oral cavity of the rhesus macaque, this is the first report of the tumor in these monkeys in the zoo gardens from the Balkan region. Experimental propagation of squamous cell malignant neoplasms in primates ended with benign lesions in rhesus macaque only. However, metastases-free neoplastic lesions were caused by fractions of the mineral oil. Squamous cell carcinomas were documented experimentally in 57 to 83 months long treatment with methylnitrosourea (1). In rodents, parenteral applications of these toxins can cause dermal changes including carcinoma (2). In our case, according to the age of the animal, the combination of the aforementioned factors could cause described carcinoma.

REFERENCES:

1. Betton G.R.: Spontaneous Neoplasms of the Marmoset (*Callithrix jacchus*). Oral and Nasopharyngeal Squamous Cell Carcinomas *Vet Pathol* 1984, 121: 193-197.
2. Fincham, J. E., Van Rensburg, S. J., Kriek, N. P. J.: Squamous Cell Carcinoma in an African Green Monkey. *Vet. Pathol.* 1982; 19:450-453.
3. Haddad, J.L., Edward J. Dick Jr., E.J., Guardado-Mendoza, R., Hubbard, G.B.: Spontaneous Squamous Cell Carcinomas in 13 Baboons, a First Report in a Spider Monkey, and a Review of the Nonhuman Primate Literature. *J. Med. Primatol.* 2009; 38(3):175–186.
4. Head, K.W., Dubielzig, R.R., Else, R.W., Misdrop, W., Patnaik, A.K., Tateyama, S., Van Der Gaag, I.: Histological Classification of Tumor of the Alimentary System of Domestic Animals. International Histological Classification of Tumors of Domestic Animals. 2nd series, Vol. X, Armed Force Institute of Pathology & C. L. Davis Foundation, Washington, DC, 2003; 27-46.
5. Nakamura, S., Sakakibara, I., Ono, F., Shibata, S., Michishita, M., Ishii, Y., Kobayashi, R., Takahashi, K., Yoshikawa, Y.: Squamous Cell Carcinoma of the Oral Cavity in an Infant Cynomologus Monkey. *Exp. Anim.*, 2000;49(3): 225-228.
6. Schmitz, H. C., Weishaupt, D., Borel, N., Padberg, B., Bürki, K.: The use of ultrasound and computed tomography for the diagnosis of a squamous cell carcinoma

of the oesophago-cardial region of the stomach in a rhesus monkey. *Lab. Anim.* 2004; 38: 92–97.

7. Ueda, M., Somura, H., Matsui, K., Matsumoto, R., Yamamoto, Y., Tamai, K.: A Squamous cell carcinoma of the oral cavity in a Francois's leaf monkey (*Trachypithecus francoisi francoisi*). *A.Z.W.M.P.* 2nd symposium and 1st workshop, Bangkok. 2006; 7-8.

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