

HAEMATO-BIOCHEMICAL PROFILE OF A MIXED BREED FEMALE NIGERIAN HORSE WITH A SUSPECTED CASE OF OSTEOSARCOMA OF THE DISTAL LIMB

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ABSTRACT

Osteosarcoma is the most common bone tumour in dogs, cats and humans. A good way of detecting health problems is through a comprehensive evaluation of the blood profile. This case report describes the haematological and serum biochemical characteristics of a mixed breed female Nigerian horse with osteosarcoma of the proximal sesamoid and phalanxes. Haematological findings indicated neutrophilia and lymphocytopenia, low packed cell volume (PCV) and haemoglobin (Hb) concentration. Biochemical analysis revealed high level of serum alkaline phosphatase (ALP) and low level of serum calcium (hypocalcemia). The observed high level of serum ALP and hypocalcemia in this reports supported many studies on the prognostic role of serum ALP and calcium in patient with osteosarcoma.

Keywords: Osteosarcoma, radiograph, Nigerian horse, haematology, serum biochemistry

INTRODUCTION

Osteosarcomas are malignant bone tumours rarely described in horses, but commonly found as primary bone neoplasia in man, dogs and cats (Cillan-Garcia *et al.*, 2010). In dogs and cats osteosarcoma is the most common bone tumour, where it originates preferentially in the metaphyseal region of long bones (Cillan-Garcia *et al.*, 2010). This case report describes the haematological and serum biochemical characteristics of a mixed breed female Nigerian horse with showing radiographic characteristics of osteosarcoma of the proximal sesamoid and phalanxes (fig. 1).



Figure 1: Lateral radiographic view of the foot showing aggressive periosteal reaction with a sun-burnst appearance on the proximal sesamoid bone (orange arrow), first phalanx (blue arrow) and second phalanx (green arrow).

Case Presentation

A 10-year-old mixed breed (Barbs, Dongola and Arab crosses) female Nigerian horse radiographically diagnosed with osteosarcoma (figs. 1) of the proximal sesamoids and phalanxes of the left forelimb was presented to the Staff Veterinarians of University of Nigeria, Nsukka Veterinary Teaching Hospital during a Clinical Ambulatory visit to Obollo-Afor horse lairag for haematology and serum biochemical assessment.

Clinical Examination

Following manual restraint with minimal discomfort by the horse handlers, five millilitres (5ml) of blood was collected aseptically from the jugular vein with the assistance of attendants. Two millilitres from the 5ml was quickly dispensed into bottles with ethylene diaminetetracetate (EDTA) for the haematological evaluation. The remaining 3ml of blood was dispensed into a plain glass test tube and allowed to clot at room temperature. The serum for biochemical determinations was separated from the clot within one hour of blood collection by centrifugation at 3,000 revolutions per minute for 10 minutes using a table centrifuge (TDL4®, B. Bran Scientific and Instruments Co., England).

Haematological and serum biochemical parameters were assessed using the established standard procedures.

RESULTS AND DISCUSSION

Haematological findings indicated neutrophilia and lymphocytopenia, erythropenia, low PCV and haemoglobin (Hb) concentration (table 1). The serum albumin, globulin, AST, urea and creatinine were within the range of the normal reference values (table 2).

Table 1: Haematological findings

Parameters	Obtained value	REFERENCE value
Packed Cell Volume (%)	27.36	31.5 – 53.5
Red Blood Cells (X 10 ⁶ /μl)	4.51	6.46 – 11.16
Haemoglobin (g/dl)	10.21	11.53 – 19.86
White Blood Cells (X 10 ⁶ /μl)	10.40	5.4 – 13.9
Neutrophils (X 10 ³ /μl)	9.12	2.05 – 8.83
Lymphocytes (X 10 ³ /μl)	1.02	1.54 – 6.79
Monocytes (X 10 ³ /μl)	0.01	0.00 – 0.15
Eosinophils (X 10 ³ /μl)	0.10	0.00 – 0.20
Basophils (X 10 ³ /μl)	0.05	0.00 – 0.20

Table 2: Serum biochemical findings

PARAMETERS	Values obtained	REFERENCE values
AST (IU/L)	78.50	74.38 – 115.06
ALT (IU/L)	9.12	3.01 – 29.43
ALP (IU/L)	112.20	34.24 – 109.76
TP (g/dl)	6.14	5.57 – 7.74
Albumin (g/dL)	3.23	2.77 – 4.06
Globulin (g/dL)	2.59	2.16 – 4.50
Calcium (mmol/L)	1.92	2.43 – 3. 10
Magnesium (mmo/L)	4.40	3.90 – 5.80
Iron (mmo/L)	11.20	10.20 – 29. 00
Creatinine (mg/L)	1.81	1.80 – 2.00

AST = Aspartate Aminotransferase, ALT = Alanine Transferase, ALP = Alkaline Phosphatase

TP = Total Protein

The biochemical analysis revealed high level of serum alkaline phosphatase (ALP) and low level of serum calcium. Serum ALP is a routine diagnostic test in clinical laboratories and is a convenient and effective biomarker for predicting the severity and outcome of osteosarcoma both in humans and animals. In humans, it has been reported that serum alkaline phosphatase (SALP) is commonly elevated in osteosarcoma patients (Hai-Yong et al., 2015). It is suggested that transformed osteoblasts in osteosarcoma would disrupt the tight control of proliferation and progressively express the genes associated with cell differentiation, causing a constantly high level of ALP (Stein et al., 1990). The observed high level of serum ALP in this case is in consonance with findings of Han *et al.* (2012), who reported that serum ALP levels increases significantly in an increased osteoblastic activity as seen in aggressive periosteal reaction associated with osteosarcoma. In addition, osteosarcoma metastasis is associated with expansion and infiltration of tumor cells, stimulating local secretion of cytokines or growth factors and causing the activation of osteoclasts, which aggravate the severity of osteolysis accompanying serum ALP elevation (Ambroszkiewicz et al., 2010). The findings in this report are also in agreement with the findings of Han *et al.* (2012) who indicated that the matrix metalloproteinases (MMPs) can be secreted by the cancer cells to dissolve extracellular matrix, which may also lead to rise of serum ALP. It is reasonable to assume that osteosarcoma progression, invasion, or metastasis would aggravate osteolysis and elevate serum ALP (Hai-Yong et al. 2015). In human, it is known that patients with osteosarcoma are commonly detected with increased serum ALP levels. The relationship between total serum ALP activity and clinical outcome of osteosarcoma patient has been recognized for over 50years (Hai-Yong *et al.*, 2015).

During the serum biochemical analysis, hypocalcemia was evident. Hypocalcemia is a rare complication of osteosarcoma both in humans and animals (Mussai et al. 2008). In humans, hypocalcemia occurs in approximately 10% of patients with metastatic cancer (Raskin *et al.*, 1973). The etiology remains unknown, and the theories range from ectopic production of a parathyroid hormone (PTH)-related peptide to the incorporation of calcium into the neoplastic lesion (Mussai et al. 2008). Hypocalcemia has been reported in human cases with multifocal osteosarcoma with associated extremely poor prognosis (Mussai *et al.*, 2008).

The observed normocromic normocytic anemia in the haematological analysis is similar to the report by Koch *et al.* (2014), in which a mare presenting with osteosarcoma of the proximal humerus showed a moderate decrease in hemoglobin and hematocrit values. This finding is also related to but differed slightly with the reports of Leite *et al.* (2019), which documented no changes in the erythrogram in a horse presenting with sinonasal osteosarcoma. Anemia is associated to chronic diseases and tumor cases both in humans and animals

(Koch *et al.*, 2014). Our findings are also related with the reports of Cançado and Chiattoni (2002), which documented that erythrogram alteration observed in chronic inflammation, infectious or tumor diseases correspond to a light to moderate normocromic, normocytic anemia. However, this report differed with the findings of Antanaitis *et al.* (2017), which reported erythropenia in a cow with osteosarcoma affecting the nasal-frontal part of the skull.

The revealed neutrophilia and lymphopenia in the haematology results in this study could be linked to a well-known relationship between systemic inflammation and tumor development (Coussens and Werb 2002). Although it is not clearly understood which mechanisms cause this relationship, some theories have been suggested that tumor-related inflammation may cause direct or indirect increases in cytokines, inhibition of apoptosis, and increases in angiogenesis (Balkwill and Mantovani, 2001). Tumor cells release granulocyte colony-stimulating factor (GCSF) that can trigger neutrophilia. Neutrophils play a role in tumor angiogenesis by producing proangiogenic factors such as vascular endothelial growth factor, matrix metalloproteinase, interleukin-8, and elastases (Balkwill and Mantovani 2001; Paramanathan *et al.*, 2014). On the other hand, lymphocytes are important in providing antitumor immunity (Kitayama *et al.* 2011). Antanaitis *et al.* (2017) reported similar lymphocytopenia in a cow with osteosarcoma of the nasal-frontal part of the skull. The neutropaenia, lymphopaenia, hypocalcemia and the radiographic findings observed in this lesion are consistent with the documented characteristics of equine osteosarcoma.

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