

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

**Kenneth C. Ogbanya, Theophilus O. Nnaji, Chinedu A. Eze**

Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Nigeria,  
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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 alterations found in a mixed breed female Nigerian horse with osteosarcoma of the proximal sesamoid and phalanxes. Haematological findings were neutrophilia and lymphocytopenia, low packed cell volume and haemoglobin concentration. Blood biochemical analysis revealed higher than normal level of serum alkaline phosphatase and lower than normal level of serum calcium (hypocalcemia). The observed high level of serum alkaline phosphatase and hypocalcemia in this report is in agreement with earlier reports on the prognostic role of serum alkaline phosphatase and calcium in both animal and human patients with osteosarcoma.*

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

Osteosarcomas are malignant bone tumours that are rare in horses, but are commonly found as primary bone neoplasia in man, dogs and cats [1]. In dogs and cats, osteosarcoma is the most common bone tumour, where it originates preferentially in the metaphyseal region of long bones [1].

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leucocytes), red blood cells (erythrocytes), and platelet or thrombocytes. Abnormally high or low counts may indicate the presence of many forms of disorders or disease, hence the reason for clinical evaluations of the blood in cases of suspected disease. A good way of detecting health problems is through a comprehensive evaluation of the blood profile – a complete blood count and serum biochemical evaluation [2]. This case report describes the haematological and serum biochemical alterations found in a mixed breed female Nigerian horse with osteosarcoma of the proximal sesamoid and phalanxes.

**CASE PRESENTATION**

A 10-year-old mixed breed (Barbs, Dongola and Arab crosses) female Nigerian horse radiographically diagnosed with osteosarcoma (Fig.1) of the proximal sesamoids and phalanxes was presented to the

Veterinary Clinicians of University of Nigeria, Nsukka Veterinary Teaching Hospital during a Clinical Ambulatory visit to Obollo-Afor horse lairage.

### **CLINICAL EXAMINATION**

Following manual restraint with minimal discomfort by the horse handlers, 5 ml of blood was collected aseptically from the jugular vein of each of the horses with the assistance of attendants. Two millilitres from the 5 ml blood was quickly dispensed into a sample bottle with ethylene diaminetetracetate (EDTA) for haematological evaluation. The remaining 3 ml 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).

The packed cell volume (PCV) was determined by the micro-haematocrit method [3]. The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method [4]. The red blood cell (RBC) and total leukocyte counts (TLC) were done by the haemocytometer method, while thin blood smear made on clean grease-free glass slides for differential leukocyte count were stained following the Leishman technique and enumerated by the meander counting method [3].

The serum biochemistry determinations followed standard procedures. Quimica Clinica Aplicada (QCA) test kits (QCA, Spain) were used for all the serum biochemistry determinations. The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method [5], while the serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method [6,7]. The serum total protein was determined by the direct Biuret method [8], while the serum albumin was determined by the bromocresol green method [9] and serum globulin was calculated as the difference between the serum total proteins and serum albumin [10]. The serum creatinine was determined by the modified Jaffe method [11].

Haematological and serum biochemical values obtained from the horse were compared with reference values in available literature [12,13]

### **RESULTS AND DISCUSSION**

Haematological findings indicated neutrophilia and lymphocytopenia, erythropania, low PCV and haemoglobin (Hb) concentration (Table 1), but other haematological parameters were within the reference interval for horses. The serum albumin, globulin, AST, urea and creatinine were within the range of the normal reference values, but there was a high level of serum alkaline phosphatase (ALP) and low level of serum calcium (Table 2), when compared to the reference values.

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, serum alkaline phosphatase (ALP) is commonly elevated in osteosarcoma patients [14]. It was 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 [15]. The observed high level of serum ALP in this case is in consonance with findings of Han et al. [16], who reported that serum ALP levels increased significantly as a result of 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 leading to serum ALP elevation [17]. The findings in this report are also in agreement with the observations of Han et al. [16] that the matrix metalloproteinases (MMPs) can be secreted by the cancer cells to dissolve extracellular matrix, which may also lead to rise in serum ALP. It is reasonable to assume that osteosarcoma progression, invasion, or metastasis would aggravate osteolysis and elevate serum ALP

[14]. In humans, 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 50 years [14].

**Table 1: Haematological findings in a horse with suspected osteosarcoma of the distal limb.**

<b>Parameters</b>	<b>Value obtained</b>	<b>Reference value [12]</b>
Packed Cell Volume (%)	27.36	31.5 – 53.5
Red Blood Cells ( X 10 <sup>6</sup> /μl)	4.51	6.46 – 11.16
Haemoglobin (g/dl)	10.21	11.53 – 19.86
White Blood Cells ( X 10 <sup>6</sup> /μl)	10.40	5.4 – 13.9
Neutrophils ( X 10 <sup>3</sup> /μl)	9.12	2.05 – 8.83
Lymphocytes ( X 10 <sup>3</sup> /μl)	1.02	1.54 – 6.79
Monocytes ( X 10 <sup>3</sup> /μl)	0.01	0.00 – 0.15
Eosinophils ( X 10 <sup>3</sup> /μl)	0.10	0.00 – 0.20
Basophils ( X 10 <sup>3</sup> /μl)	0.05	0.00 – 0.20

**Table 2: Serum biochemical findings in a horse with suspected osteosarcoma of the distal limb.**

<b>PARAMETERS</b>	<b>Values obtained</b>	<b>Reference values [13]</b>
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

Hypocalcemia was evident in the horse with suspected osteosarcoma. Hypocalcemia is a rare complication of osteosarcoma both in humans and animals [18]. In humans, hypocalcemia occurs in approximately 10% of patients with metastatic cancer [19]. 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 [18]. Hypocalcemia has been reported in human cases with multi-focal osteosarcoma with associated extremely poor prognosis [18]. Figure 1: Lateral radiographic view of the foot of a horse with suspected osteosarcoma of the limb, showing aggressive periosteal reaction with a sun-burnst appearance on the proximal sesamoid bone, first and second phalanxes (arrows).

The findings of lower than normal PCV, haemoglobin concentration and red blood cell counts in the horse being reported on in this case concurs with thereport by Koch et al. [20], 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. [21], which documented no changes in the erythrogram in a horse presenting with sinonasal osteosarcoma. Anemia is

associated with chronic diseases and tumor cases both in humans and animals [20]. Our findings are also related with the reports of Cançado and Chiattonne [22], which documented that erythrogram alterations observed in chronic inflammation, infectious or tumor diseases correspond to a light to moderate normochromic, normocytic anemia, and that of Antanaitis et al. [23], which reported erythropenia in a cow with osteosarcoma affecting the nasal-frontal part of the skull.

The findings of neutrophilia and lymphopenia in the horse being reported on could be linked to a well-known relationship between systemic inflammation and tumor development [24]. Although it is not clearly understood which mechanisms cause this relationship, some theories have suggested that tumor-related inflammation may cause direct or indirect increases in cytokines, inhibition of apoptosis, and increases in angiogenesis [25]. Tumor cells release granulocyte colony-stimulating factor that can trigger neutrophilia. Neutrophils play a role in tumor angiogenesis by producing pro-angiogenic factors such as vascular endothelial growth factor, matrix metalloproteinase, interleukin-8, and elastases [25, 26]. On the other hand, lymphocytes are important in providing antitumor immunity [27]. Antanaitis et al. [23] reported similar lymphocytopenia in a cow with osteosarcoma of the nasal-frontal part of the skull.



**Figure 1: Lateral radiographic view of the foot of a horse with suspected osteosarcoma of the limb, showing aggressive periosteal reaction with a sun-burnst appearance on the proximal sesamoid bone, first and second phalanxes (arrows).**

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