

Clinicopathological and imaging features of hypertrophic osteopathy in dogs

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Abstract

Hypertrophic osteopathy (HO) is a diffuse periosteal reactivity of long bones and of poor prognosis as most cases died after short time or euthanized at the time of diagnosis. Early diagnosis is greatly important. Therefore, the present study aimed to investigate the clinical and different imaging features of HO in 12 dogs and hematological and biochemical changes. Twenty-two dogs were included in this study; 10 apparently healthy dogs were used as controls, and 12 dogs were diagnosed with HO. Diagnosis of HO-affected cases was based on a range of clinical, laboratory, and histopathological examinations as well as different imaging techniques. Two forms of HO were diagnosed: pulmonary (n=5) and extrapulmonary (n=7) HO. In pulmonary HO, pneumonia was recorded in five dogs. However, in extrapulmonary HO, mammary gland tumors and skin tumors were recorded in five and two dogs, respectively. Symmetrical non-edematous soft tissue swelling at the lower parts of the limbs with intensive bone proliferation and periosteal reactivity in the tubular long bones was observed. Anemia and leukocytosis, mainly lymphocytosis 50% or neutrophilia 45%, and elevated serum globulin, alkaline phosphatase, and C-reactive protein levels were the significant associated laboratory findings. The use of different diagnostic techniques was greatly important in the diagnosis of HO in dogs and provided information on the prognosis of such cases.

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Introduction

Hypertrophic osteopathy (HO) is a rare progressive syndrome. It has been most commonly reported in humans (1) and dogs (2-5). Moreover, other animal species have been affected, including horses (6), cows (7), sheep (8), cats (9) and various species (10). Most forms of HO are secondary to intrathoracic masses, either neoplastic or infectious, which is known as pulmonary HO (11) or less commonly, intra-abdominal masses (extrathoracic alterations) (12). The primary form of HO has been observed in humans as pachydermoperiostosis (13). As most HOs developing in dogs are associated with thoracic masses, the disease can be known as pulmonary HO (1). The characteristic signs of HO are symmetrical non-edematous swellings of the soft tissue

that develop bilaterally and accompanied by diffuse periosteal growth at the outer aspect of the diaphysis of the long bones without interspersions of the cortical bone. These bone changes affect the distal parts of the forelimbs with mild-to-severe lameness (1,4). The disease prognosis is highly dependent on the main cause of HO. To obtain good results, the primary cause of HO should be treated with tumor excision, chemotherapy, radiotherapy, or infection therapy. In dogs, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) may be suitable to provide palliative care (14). Despite these treatment options, the prognosis of HO caused by neoplastic pulmonary lesions is extremely poor, and euthanasia of the animal is preferable than surgical interference (14).

Because HO is a rare progressive syndrome, many cases may not be diagnosed properly at early stages. Therefore, the present study aimed to investigate the clinical and different imaging features, including radiography, computed tomography (CT), and ultrasonography, of HO in dogs and hematological and biochemical changes.

Materials and methods

Animals

Twenty-two dogs were included in the present study; 12 dogs of different breeds, age, and sex were admitted to the Clinic of Faculty of Veterinary Medicine, Zagazig University, Egypt, with a history of loss of appetite, weight loss, lethargy, and lameness. Two male dogs were admitted with skin mass lesions, 5 female dogs with mammary gland mass lesions, and 5 dogs (2 males and 3 females) with respiratory manifestations. Moreover, 10 apparently healthy dogs of the same breed (5 German Shepherd dogs and 5 mixed breed dogs) and both sexes (5 males and 5 females) were used as controls.

Clinical examination

All dogs were subjected to thorough clinical examination as previously described (15). Inspection of the animals' stance and motion was performed to determine the affected limb. Palpation of limb swellings in addition to passive and active movements of the joints were applied. Rectal body temperature, pulse rate, respiratory rate, and mucous membrane were examined.

Radiographic examination

Standardized mediolateral and anteroposterior radiographs of the limbs and ventrodorsal radiographs of the chest were performed for all dogs using an X-ray machine (Pox-300 BT, Toshiba, Rotanode™, Japan) with 55–70 kV, 100 MA, and 6.3 MA exposure factors. The limb radiographs were investigated for periosteal reactivity of the long bone and joint involvement. Moreover, chest radiographs were investigated for intrathoracic radiopacity.

Computed tomographic examination

The CT of the limbs and were performed using CT scanning device (Hitachi, USA, multislice 16 scanner) with 120 kV and 200 MA to the affected dogs to investigate the periosteal reactivity and pulmonary lesions, respectively.

Ultrasonographic examination

Abdominal ultrasonography of the affected dogs was performed after application of ultrasound coupling gel using an ultrasound machine (SonoScape A5V, China) connected with 5- and 6-MHz transducers as described previously. The acoustic window of each organ was selected as described previously (16). Any abnormalities during examination were reported.

Hematological and biochemical analysis

Two blood samples were collected through the cephalic vein of each dog. The first one with ethylenediamine tetraacetic acid for hematological examination using automated cell counter F-820 (Sysmex Co. Ltd, Kobe, Japan): red blood cell count, hemoglobin concentration, packed cell volume, white cell count, and platelet count were selected. The second blood sample was plain without anticoagulant to determine serum total protein, albumin, globulin, blood urea nitrogen (BUN), calcium, and inorganic phosphorus levels. Enzymatic activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured spectrophotometrically by standard procedures using diagnostic Zrt. Commercial kits (BioMerieux, Egypt). Immunological examinations for C-reactive protein (CRP), rheumatoid factors (RF), antinuclear antibodies (ANA), and tumor necrosis factor (TNF) were performed using available kits for humans according to manufacturer's instructions. Serum alpha-fetoprotein (AFP) concentrations were determined using sandwich enzyme-linked immunosorbent assay for canine AFP. Carcinoembryonic antigen (CEA) level was determined using a radio-indicative method-immunoradiometric analysis with the use of commercially available kits for humans.

Surgical management and prognosis of HO-affected dogs

Surgical excision of the skin and mammary gland mass lesions was performed aseptically under the effect of general anesthesia (2.5% thiopental sodium, 20mg/Kg B Wt, I/V, Epico Co. 10th of Ramadan City, Egypt). The HO-affected dogs with respiratory manifestations were died after variable periods from 2 to 8 months from admission. These dogs were subjected to standardized necropsy procedures and representative tissue specimens from long bones and thoracic and abdominal organs were collected for histopathological examination.

Histopathological examination

The excised masses and tissue specimens of died dogs were collected and fixed immediately in 10% neutral buffered formalin solution for 72h. Then the bone specimens were decalcified in 10% ethylene diamine tetraacetic acid (EDTA), which was changed every 3 days for 4 weeks at room temperature. After decalcification the bone samples were dehydrated in a series of graded concentrations of ethanol from 70% to 95%, whereas the soft tissue samples started at 30% and were then embedded in paraffin. The samples were cut into 5 µm thick sections and stained with hematoxylin and eosin (H&E) according to the manufacturer's protocol and then examined microscopically (17-20).

Statistical analysis

All data were statistically analyzed using SPSS (version 17, USA). Analysis of variance with Duncan's post hoc test was used to determine the significance level between two groups. The results were expressed as means \pm standard error. A $P < 0.05$ indicated statistical significance.

Results

Based on clinical findings, imaging techniques and histopathological findings, the twelve dogs diagnosed with HO, five dogs with pneumonia, five dogs with mammary gland tumors and two dogs with Skin tumors.

History and clinical findings

The age of the affected dogs ranged from 1.5 to 7 years. The most affected breed was German Shepherd (six dogs), followed by mixed breeds (four dogs) and Black Jack (two dogs). Female dogs were mostly affected than male ones ($n = 8$ and 4 , respectively). All dogs were reported with anorexia, loss of body weight and lethargy. Pyrexia and ocular discharge of mucopurulent nature were reported in five dogs with respiratory manifestations. The reported respiratory manifestations were abnormal lung sounds, tachypnea, dyspnea, and cough. Lameness of one or more limbs were noticed at standing and during motion in nine dogs. Symmetrical non-edematous soft tissue swellings affecting the lower parts of the limbs were observed (Figure 1).



Figure 1: Clinical findings of the HO-affected dogs. (a) Symmetrical non-edematous swellings of the lower extremities (black arrows) and digital clubbing (red arrows). (b) Mucopurulent ocular discharge (black arrow) and anemic mucous membrane of the mouth (blue arrow). (c) Mammary gland tumor. (d) Skin tumor at the palmar aspect of the right forelimb.

Restricted flexibility of the involved joints was also observed in four dogs. Mammary gland tumors were recorded in five dogs (Figure 1c). Moreover, skin tumors were recorded in two dogs at the lower parts the limbs (Figure 1).

Radiographic and CT findings

Radiographs and CT images of the limbs of the affected dogs showed severe degenerative condition with periosteal proliferative newly formed bone at the proximal and distal metaphysis and diaphysis of the long tubular bones. All long bones were involved in five dogs, and the radius/ulna and tibia/fibula were involved in four dogs, while the radius/ulna only was involved in three dogs (Figure 2). The joint articular surfaces were not involved and appeared normal without degeneration.

The radiographs and CT images of the chest of pulmonary HO-affected dogs showed multiple patches of radiopacity in the pulmonary tissue of five dogs, representing inflammatory pulmonary condition (Figure 3).

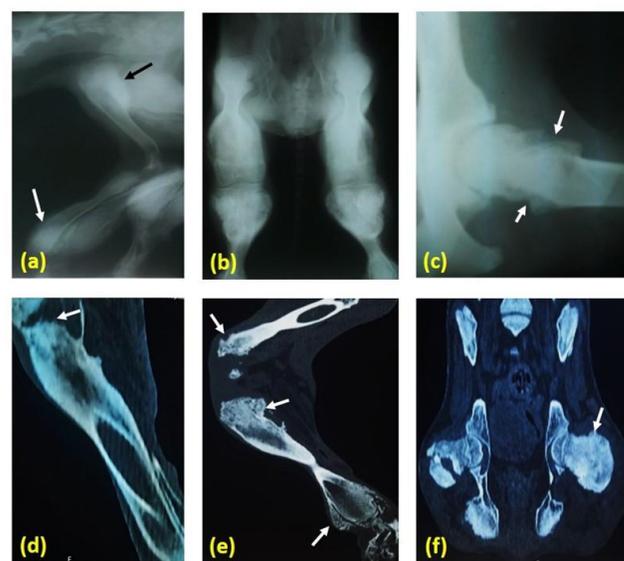


Figure 2: Limb radiographs and CT imaging of the HO-affected dogs. (a) Mediolateral radiograph of the forelimb showing enlarged proximal and distal extremities of the humerus and radius/ulna with irregular periosteal reaction (arrows). (b) Ventrodorsal radiograph of the pelvis and hind limbs showing irregular enlargement of the proximal and distal extremities of the femur and tibia/fibula. (c) Ventrodorsal radiograph of the hip joint showing periosteal projections at the femoral neck (arrows). (d) CT image of the humerus showing irregular periosteal reaction at the proximal extremity (arrows). (e) CT image of the hind limb showing periosteal reactions at the distal extremity of the femur and proximal and distal extremities of the tibia (arrows). (f) CT image of the hip joints showing periosteal reactivity at the femoral neck (arrow).



Figure 3: Chest CT image of a 1.5-year old German Shepherd dog. The lung showing patches of radiopacity in the pulmonary tissue (arrow).

Ultrasonographic findings

Abdominal ultrasonography of HO-affected dogs revealed normal homogenous echogenic characters of the hepatic parenchyma, spleen and kidneys. Gastric and intestinal tissues were normal. There were no tumor lesions in the mesenteric lymph nodes.

Hematological and biochemical findings

The hematological and biochemical findings of HO-affected animals and normal dogs are shown in Table 1. The most common hematological abnormalities in HO-affected dogs were anemia and leukocytosis with lymphocytosis and neutrophilia when compared with the normal dogs. Erythrocyte sedimentation rate at the first hour was higher in HO-affected dogs. The serum globulin level was highly significant ($P < 0.001$) in HO-affected dogs at the expense of serum albumin level. Regarding serum enzymology, although the serum AST, ALT, and LDH activities were increased in HO-affected dogs, there were no significant differences with the normal ones. The activity of ALP and BUN level were highly significant ($P < 0.001$ and $P < 0.05$, respectively) in HO-affected dogs than in the normal ones. The serum AFP level was mildly increased in HO-affected dogs with skin tumors. The serum CEA level of two HO-affected dogs with mammary gland tumors was 6 and 9 times higher than those of the normal dogs. Despite the higher TNF levels in all HO-affected dogs, it was highly significant in HO-affected dogs with pneumonia. The serum CRP levels in HO-affected dogs were significantly higher than normal dogs ($P = 0.007$), while the serum RF and ANA levels in HO-

affected dogs were within normal range when compared to those in the normal dogs.

Postmortem and histopathological findings

During necropsy, enlargement of the extremities of the long bone extending to the diaphysis was observed. Moreover, the lungs showed consolidation of many lobules. The abdominal organs were normal and did not show any abnormalities either in shape or in size. In histopathological examination, mineralized newly formed bone spicules with benign-appearing osteoblast and osteocytes at the periosteum parallel to the main bone cortex at the level of the long bone diaphysis and metaphysis were observed (Figure 4a). The histopathological sections of the lungs showed fibrinous pneumonia with lymphocyte, neutrophil, and macrophage infiltration. Congestions, extensive hemorrhages, and edema associated with areas of consolidation were also visualized (Figure 4b). The skin masses revealed epidermal layer tumor with capsulated dermal nodules, which formed from whorls of mature and immature fibroblasts (Figure 4c). While mammary gland masses showed simple-type solid carcinoma (Figure 4d).

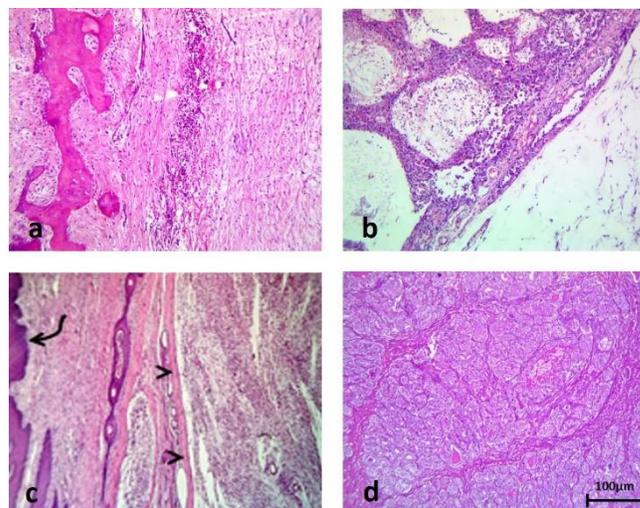


Figure 4: Histopathology of the HO-affected dogs. (a) The bone parenchyma showing mineralized newly formed bony spicules with benign-appearing osteoblast and osteocytes among severely dilated capillaries. (b) Lung tissue revealed fibrinous exudation infiltrated with lymphocytes, neutrophils, and macrophages. (c) Skin mass section showing epidermal layer of the skin tumor (curved arrow) with capsulated dermal nodule (black arrow heads) and some cells showing myxoid appearance with presence of cells of different shapes and sizes (open arrows) surrounded by glycoprotein matrix (red star) and cellular process of tumor cells (red arrow heads) criss cross each other. (d) Simple-type solid carcinoma of the mammary gland tumor consists of solid tumor cell masses formed by epithelial and myoepithelial cells.

Table 1: Hematological and biochemical findings of the control and HO-affected dogs

Items	Control dogs			HO affected dogs			p value
	Mean±SD	Min	Max	Mean±SD	Min	Max	
RBCs (x10 ¹⁰ /mm ³)	6.21±0.31	5.52	7.30	3.15 ± 0.31	2.31	3.87	< 0.001*
PCV (%)	44.40±1.57	40.00	49.00	27.20± 1.28	23.00	30.00	< 0.001*
Hb (g/dl)	14.32±0.33	13.30	15.30	9.28±0.53	8.10	10.80	< 0.001*
WBCs (x10 ³ /mm ³)	9.86±0.21	9.40	10.49	19.38±3.72	10.32	29.27	0.034*
Neutrophils (%)	53.53±2.52	45.23	60.00	51.61±12.48	8.07	83.13	0.884
Lymphocytes (%)	39.22±3.11	29.25	47.70	42.04±13.01	8.87	87.00	0.838
Monocytes (%)	2.66±0.24	2.05	3.30	3.28±0.27	2.57	4.00	0.126
Esinophils (%)	2.38±0.58	1.00	4.20	2.67±0.78	1.08	5.00	0.771
Basophils (%)	0.20±0.20	0.00	1.00	0.40±0.24	0.00	1.00	0.545
Platelets (x10 ³ /mm ³)	279.60±41.58	185.00	390.00	368.20±56.25	213.00	534.00	0.241
ESR (mm/hr)	1.80±0.97	0.00	5.00	15.40±9.06	2.00	50.00	0.174
Tp. (g/dl)	7.25±0.19	6.47	8.03	6.96±0.21	6.22	7.77	0.330
Alb. (g/dl)	4.24±0.09	3.87	4.53	2.97±0.08	2.71	3.22	< 0.001*
Glo. (g/dl)	3.02±0.13	2.63	3.62	3.62±0.12	3.30	4.22	< 0.001*
AST (iu/l)	15.54±1.40	10.75	22.52	28.72±7.57	12.94	69.56	0.109
ALT (iu/l)	16.72±3.37	7.98	33.25	60.35±23.53	13.50	168.70	0.088
ALP (iu/l)	92.38±4.89	67.45	107.50	472.13±39.47	199.00	620.00	< 0.001*
LDH (iu/l)	202.50±10.51	160.00	253.00	292.41±27.74	231.22	348.28	0.177
BUN (mg/dl)	16.25±2.29	7.36	25.28	40.19±9.26	17.11	82.39	0.025*
Ca (mg/dl)	10.43±0.19	9.85	11.18	10.44±0.22	9.82	11.52	0.983
Ph (mg/dl)	6.21±0.15	5.82	6.77	6.05±0.17	5.42	6.93	0.474
AFP (ng/ml)	0.88±0.31	0.05	2.83	5.23±3.13	0.08	20.23	0.189
CEA (ng/ml)	0.08±0.01	0.03	0.13	0.19±0.10	0.01	0.71	0.279
TNF α (ng/ml)	0.003±0.002	0.001	0.03	3.42±2.20	0.02	15.88	0.142
CRP (ng/ml)	0.05±0.01	0.01	0.12	1.69±0.53	0.17	4.80	0.007*
RF (ng/ml)	0.92±0.25	0.19	2.43	0.95±0.30	0.16	2.18	0.928
ANA (u/ml)	0.15±0.04	0.05	0.36	0.17±0.05	0.07	0.46	0.707

*Significant difference between the HO affected dogs and the control dogs.

Discussion

HO is a progressive syndrome that rarely develops in different animal species. Its diagnosis needs many imaging techniques for detection of the main cause and form of HO in affected animals, either primary or secondary form in addition to the clinical signs. As dogs have the second highest incidence after humans (2,3), therefore, the present study focused on the clinical and hematological findings of HO in dogs in addition to imaging features using different imaging techniques, such as radiography, CT, and ultrasonography. Secondary HO in dogs, the most common form, commonly results from intrathoracic lesions, which is called pulmonary HO, or of less extent resulted from intra-abdominal lesions, which is called extrapulmonary HO (11,12). In the present study, pulmonary HO was reported in five dogs, and extrapulmonary HO was reported in seven dogs.

In secondary HO, information about breed, sex, and age distribution have little meaning. However, HO mostly develops in middle- to old-aged animals because of the association with neoplasia (1). In the present study, the age

of the affected dogs ranged from 1.5 to 7 years, and German Shepherd dogs were mostly affected (n=6). This might be due to the interest of individuals in our population to keep this dog breed. Moreover, women were more commonly affected than men. This might be because mammary tumors that might cause metastatic lung tumors might consequently lead to HO (1).

Symmetrical non-edematous swellings of the lower portions of the extremities were the characteristic clinical finding in HO diagnosis (1) that might be limited to the forelimbs (2). Mucopurulent ocular discharges might be observed in HO-affected dogs (3,21). Respiratory manifestations were correlated with pulmonary HO (21). Similar findings were reported in the present study.

Radiography and CT of the limbs are the first imaging modalities used to diagnose HO cases, even in symptomatic patients (22). Radiopaque periosteal reaction was observed in radiographic and CT images of the long bones. Periosteal proliferations were either irregularly shaped perpendicular to the cortex or smooth shaped parallel to the cortex (1). Although the joints were not affected, the joint motion might become diminished due to periarticular soft tissue swelling

(1). Chest radiographs and CT images showed multiple patches of radiopacity in the pulmonary tissue of five cases, representing inflammatory condition of the lungs. Similar findings were previously reported (3,14). Ultrasonography is a safe, noninvasive, reliable, and available diagnostic tool for human and small animal practices (23). It was used for examination of the abdominal cavity for any abnormalities in HO-affected dogs.

Regarding laboratory findings, anemia have been reported in HO-affected dogs with hematocrit levels ranging from 26.3% to 38.5% (3,21). It is a common paraneoplastic syndrome, resulting from inflammation, immune-mediated and microangiopathic hemolysis, and blood loss (24). Leukocytosis was also recorded in HO-affected dogs (25). These findings were reported in the present study. A nonsignificant increase in platelet count has been reported. On the contrary, a significant increase in mean platelet count in HO-affected animals compared to that in control ones was recorded (2).

The recorded hypoalbuminemia was similar to those reported previously where they owed hypoalbuminemia to inflammation, malnutrition, and undiagnosed renal losses or secondary to elevated globulin levels (3). Meanwhile, hyperglobulinemia is the product of inflammation secondary to HO or primary neoplastic disease. An elevated serum globulin level in affected animals indicates that the immune system is working overtime, potentially battling infection, inflammation, and/or cancer. The serum ALP levels help in the diagnosis and follow-up of dogs affected with HO. Elevation of serum ALP level might be either attributed to enhanced osteoblastic activity as part of the progression of HO or secondary to bone metastases (3,25). Serum CRP level is an objective and quantitative marker of inflammation (26). Its measurement has been shown to be clinically useful in diagnosing and monitoring systemic inflammatory diseases (27).

In humans, the serum AFP level was used in early diagnosis of hepatocellular carcinoma and its concentration was correlated with the size of the tumor masses that might be due to increased rate of necrosis at the center of the tumor masses (28). However, its serum level was mildly elevated in some dogs with hepatic and non-hepatic diseases, even in those without tumor (29). In our study, a mild elevation of the serum AFP level was reported in two HO-affected dogs with skin tumors. The serum CEA level was highly significant in five HO-affected dogs with mammary gland tumors. Concerning the serum RF and ANA levels, published guidelines in human medicine suggested that all patients with suspected idiopathic pulmonary fibrosis should be screened with RF, cyclic-citrullinated peptide, and ANA tests (30). Circulating ANAs are commonly present in human systemic autoimmune disease and other systemic rheumatic diseases and in dogs (31).

Conclusions

Symmetrical non-edematous swellings of the lower parts of the extremities were the characteristic sign of HO in dogs. Intrathoracic lesions were the incorporated causative agent predisposing dogs to HO. The use of different imaging techniques is helpful in the diagnosis of HO and determination of its type, either pulmonary or extrapulmonary. The survival rate of HO-affected dogs was extremely limited and depended on the severity and stage of the disease.

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Conflicts of interest

The authors declare no conflict of interest.

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السمات السريرية والمرضية والتصويرية للاعتلال العظمي الضخامي في الكلاب

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الخلاصة

اعتلال العظم الضخامي هو تفاعل شديد منتشر في الغشاء المبطن للعظام الطولية وتعتبر من الحالات غير المستجيبة للعلاج لأن معظم الحالات تتوفي بعد وقت قصير أو تعدم أثناء التشخيص. فالتشخيص المبكر لهذه الحالات مهم للغاية. لذلك، هدفت الدراسة الحالية إلى فحص الأعراض السريرية وباستخدام الأشعة التصويرية المختلفة بالإضافة اللي التغيرات الدموية والكيمياء الحيوية في عدد ١٢ كلباً مصاباً بالاعتلال العظمي الضخامي. تم استخدام عدد ٢٢ كلباً في هذه الدراسة منهم عدد ١٠ كلاب سليمة سريريّاً كمجموعة حاكمة وعدد ١٢ كلباً مصاباً بالاعتلال العظمي الضخامي. اعتمد التشخيص على الفحص السريري والمعملي والفحص النسيجي بالإضافة الأشعة التصويرية المختلفة. تم تشخيص نوعين من الاعتلال العظمي الضخامي: النوع الرئوي في عدد ٥ حالات والنوع خارج الرئة في عدد ٧ حالات. تم تسجيل الالتهاب الرئوي في الحالات المصابة بالنوع الرئوي بينما تم تسجيل الأورام التذبية في عدد ٥ حالات وأورام الجلد في حالتين من الحالات المصابة بالنوع خارج الرئوي. لوحظ تورم متناظر للأنسجة الرخوة غير المتوزمة في الأجزاء السفلية من الأطراف مع تكاثر عظمي مكثف وتفاعل شديد منتشر في الغشاء المبطن للعظام الطولية الأنثوية. كانت النتائج المعملية المصاحبة عبارة عن فقر في الدم وزيادة في كريات الدم البيضاء، وزيادة في معامل الالتهابات. يعتبر استخدام وسائل التشخيص المختلفة مهماً للغاية في تشخيص الاعتلال العظمي الضخامي في الكلاب ويفيد في دراسة تطور الحالة المرضي ومدى استجابتها للعلاج فيما بعد.