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A Typical Case of Chronic Myeloid Leukemia (CML) in A Native Shepherd Dog in Duhok Province, Iraq

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ABSTRACT

A typical case of a 7-years-old Kurdish male shepherd dog was referred with two weeks history of lethargy, restlessness, anorexia and severe emaciation. Physical examination of the dog revealed generalized lymphadenopathy, strong pale mucous membrane, systolic murmur of the heart, vomiting, bloody diarrhea and fever. Hematological abnormalities indicated moderate to marked leukocytosis characterized by 89% neutrophilia with a left shift to progranulocytes and 1.6% presumptive myeloid blasts, marked thrombocytopenia, marked non-regenerative normocytic hypochromic anemia and dysplasia in platelets and neutrophils. The histopathological examination revealed excessive infiltration with neoplastic myeloid cells that invaded all the internal organs. Bone marrow analysis revealed marked hypercellularity with a predominance of immature cells, marked myeloid 6.2%, dysplasia of neutrophils lineage and rare erythroid progenitors and numerous megakaryocytes. Enlargement of superficial and visceral lymph nodes and internal body organs, especially liver and spleen were observed in the autopsy after animal euthanasia. Our results confirmed that this dog was suffering from chronic myeloid leukemia. Statistically, there was a significant difference ($P < 0.05$) between the mean value of myeloid blasts in peripheral blood and bone marrow. But, no significant difference ($P > 0.05$) was found between the mean values of leukocytic ratio in peripheral circulation and bone marrow. This is the first case report study that has been recorded in a native shepherd dog in a rural area around Duhok province/ Iraq.

Case Study:
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INTRODUCTION

Chronic myeloid leukemia (CML), also known as “chronic myelogenous leukemia," is a form of leukemia that starts in the blood-forming cells of the bone marrow. When a pluripotent steam cell undergoes malignant transformation and clonal myeloproliferation, it leads to striking overproduction of immature granulocytes in the bone marrow and accumulating these cells in the blood (**Thrall *et al.*, 2012**).

The term ‘chronic’ in chronic myelogenous leukemia indicates cancer tends to progress more slowly than acute forms of leukemia. CML is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia (Ph) chromosome or t (9; 22) translocation (BCR-ABL). A similar translocation has been described in Raleigh's dogs **(Cortes *et al.*, 2012; Breen and Modiano, 2008).** This type of leukocyte cancer commonly occurs in middle and old-aged dogs and resembles the cat and human type of leukemia.

The purpose of this case report is to describe clinicopathological findings and provide useful information for the accurate diagnosis of chronic myeloid leukemia in dogs.

MATERIIALS AND METHODS

Clinical History:

A 7-year-old native male shepherd dog weighing 40.5 kg was presented to the clinical pathology laboratory, College of Veterinary Medicine/ University of Duhok, Iraq, 2021. The dog suffered from lethargy, restlessness, inappetence, vomiting, and loss of body weight for two successive weeks. The dog was treated with several antibiotics including Penicillin, Streptomycin and Amoxicillin. Several doses of Dexamethasone and different types of Multivitamins were given during a period of one month.

Physical examination revealed severe dyspnea, Labor respiration, tachycardia, very pale mucus membranes, severe emaciation, dehydration, peripheral lymphadenopathy, fever, bloody diarrhea and Systolic heart murmur during thoracic auscultation. The abdomen was distended and internal organs were palpable because of Organomegaly. Because of severe abdominal distention and severe critical health status, the diseased animal was euthanized by injecting with an overdose of Pentobarbital sodium solution via cephalic vein. A post-mortem examination was applied according to **Joiner *et al*., (1976).**

Laboratory examination:

Complete Blood Count (CBC):

A blood sample (5 ml) was taken from the cephalic vein and analyzed by using an automated hematology analyzer (Mindray/ BC-2800 machine, Shenzhen 518057/ China) to evaluate the blood components (TLC, RBCs count, Hct/PCV, Hb concentration, Reticulocyte count and platelets count) and blood indices (MCV, MCH and MCHC), **(Christina *et al*., 2017).**

Peripheral Blood Film (PBF) Examination:

For morphological evaluation of leukocytes, erythrocytes and thrombocytes, thin smears of circulating blood were prepared, dried and immersed in absolute methanol for 5 minutes for fixation. The blood smear slides were dried after fixation. A 1 in 10 dilutions of Giemsa stain in distilled water was prepared and poured into a staining jar. The slides were immersed in the stain for 30 minutes, washed, dried again, and examined under an oil immersion lens **(Mayer and Harvey, 2004).**

Histopathological examination:

Samples of bone marrow, lung, liver and kidney were fixed in 10% of neutral buffered formalin and dehydrated by different ethanol concentrations. The samples were embedded in paraffin, sectioned at 5 µm and finally stained with Hematoxylin and Eosin (H&E) for evaluation and examination under a light microscope (x40) as previously described **(Bancroft and Stevens, 2020).**

Statistical analysis:

Unpaired student t-test was used to compare the mean values of neoplastic myeloid cells and leukocytes ratio in peripheral blood smears and bone marrow samples through applying SPSS statistics software (IBM, version 19.0).

RESULTS

Results of the hemogram of the diseased native dog showed severe microcytic hypochromic anemia, marked leukocytosis, and marked thrombocytopenia (Fig. 1). The severe microcytic hypochromic anemia was accompanied by nucleated erythrocytes and Howell jolly bodies (bluish spherical bodies) in the blood smears. A marked leukocytosis due to severe neutrophilia was recorded. Degenerative shift to the left up to the progranulocyte stage with low numbers of myeloblasts; mononuclear cells showing oval to round nucleus with fine chromatin and scanty pale blue cytoplasm (Table, 1) were also demonstrated. The thrombocytopenia was associated with the presence of dysplastic platelets and micro-megakaryocytes in the blood smear (Fig. 2).

At necropsy, enlargement of superficial and visceral lymph nodes was recorded. Spleen was greatly enlarged, dark red with different sized yellow foci scattered everywhere. The liver was slightly enlarged with degenerated texture. The lung was enlarged, flabby, pale in color, easily distracted into small particles during manipulation with a bad odor (Fig. 3). Excessive amount of reddish-brown marrow revealed was seen within the femur, ribs and vertebral bone.

Examining tissue from bones after an autopsy showed a hyperplastic bone marrow, numerous segmented and band neutrophils scattered among primitive mononuclear cells. Granulocytes comprise approximately 92% of the cellular population. A myeloid blast comprises approximately 6.2% of the differential leukocyte count (Table 2). There were also large numbers of megakaryocytes. Dysplasia was also noted in neutrophils lineage and rare in erythroid progenitors (Fig. 4).

Table 1: Laboratory Hematology Results

Blood Components	Results	Reference Rages
Red Blood Cells (RBSs)	2.6 x 10^6/ µl	4.8 – 9.3 x 10^6/ µl
Hemoglobin (Hb)	3mg/ dl	12.1 – 20.3 g/dl
Hematocrit (Hct)	12%	37-55%
Reticulocytes	0.6%	0 – 1.1%
Total Leukocyte Count (TLC)	112 x 10^3/ µl	4.0 – 15.5 x 10^3
Mean Corpuscular Volume (MCV)	46.5/ f*l*	60 – 80/ f*l*
Mean Corpuscular Hemoglobin Concentration (MCHC)	25gm/dl	30 - 38 gm/dl
Differential Leukocyte Count (DLC)	89%	40 – 75%

Table 2: Percentage score of Myeloid blasts cells and leukocytic cells in peripheral blood and bone marrow

Cell's position	Myeloid blasts %	Leukocytic cells %
Bone marrow	6.2 %	92 %
Peripheral blood	1.6 %	89 %

Histological examination of liver, lung and kidney revealed excessive infiltration with neoplastic myeloid cells (reduced cytoplasmic proportion to nuclear size), large number of megakaryocytes and its precursors and blast cells (Fig. 5)

Fig.1: Severely debilitated native dog with abdominal distention (organomegaly).

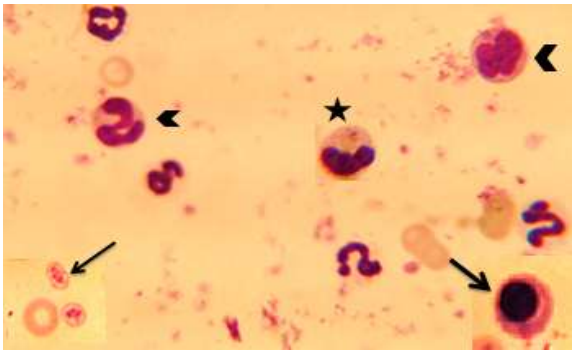

Fig. 2: Peripheral blood smear shows dysplastic and segmented neutrophils (small arrowhead, binuclear metamyelocyte (big arrowhead), metamyelocyte (star), dysplastic platelets (thin arrow) and a presumptive myeloid blast (thick arrowhead), Giemsa stain x100.

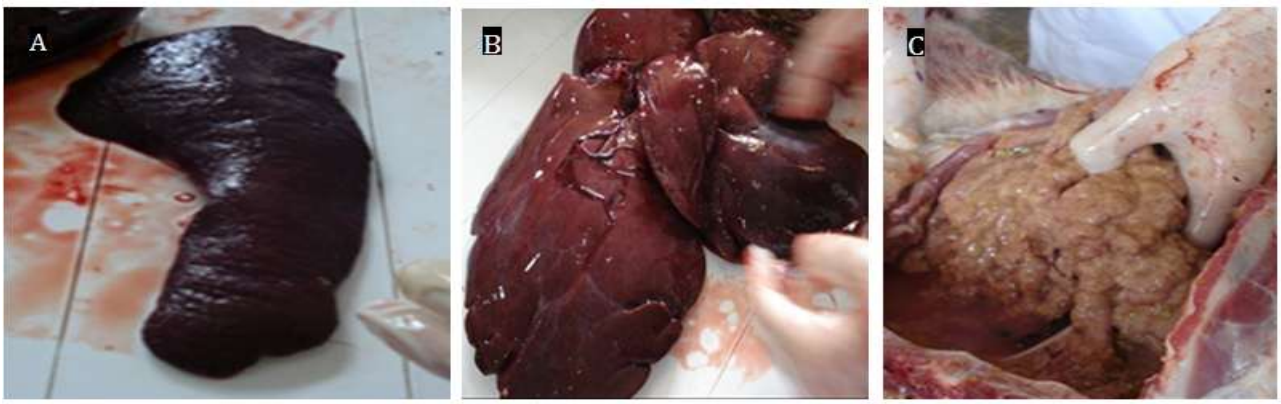

Fig. 3: Enlargement and Degeneration in texture of Internal body organs including A: Spleen, B: Liver, C: lung.

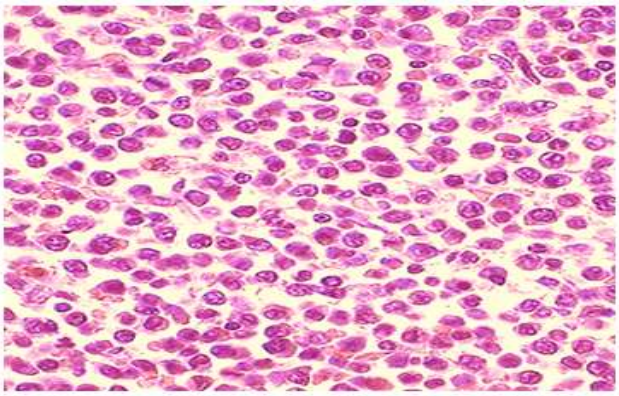

Fig. 4: Bone marrow showing the histologic characteristics of CML including hyper-cellularity and intensive granulocytic proliferation, especially in neoplastic megakaryocytes and megakaryocytic blast cells (H & E staining, x under high power).

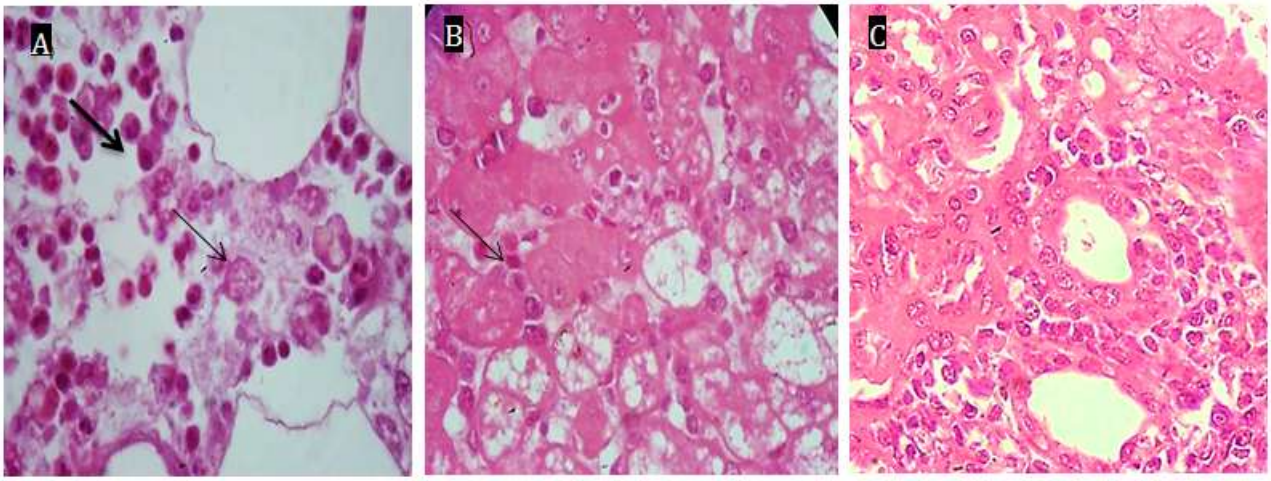

Fig. 5: A high power histopathological staining (H&E) of chronic myeloid leukemia in A/ Lung: shows a predominant population of blasts (thick arrow) with numerous scattered neoplastic megakaryocytes (thin arrow). B/liver: Shows neoplastic megakaryocytes noted in sinusoidal spaces and many blast cells evident in the periportal area (arrow). C/ Kidney: Shows excessive infiltration of neoplastic myeloid cells scattered everywhere.

DISCUSSION

Chronic Myeloid Leukemia (CML) is a myeloproliferative disease characterized by abnormal proliferation of the myeloid cell population and results from a single pluripotent stem cell in the bone marrow. In dogs, the CML is similar to Chronic Lymphocytic leukemia (CLL) in that it is a disorder of middle and old ages. This case was an old-aged dog that is compatible with a previous report **(Ji-Yan *et al.*, 2005).** Routine physical examination revealed fever, dyspnea, pale mucous membrane, tachycardia, systolic heart murmur, bloody diarrhea, dehydration, severe emaciation and generalized lymphadenopathy **(Hee-Myung *et al.*, 2006)**.

In the present work, several parameters were used for diagnosis of the CML including complete blood count, histopathological examination, morphological examination of a peripheral blood smear, and bone marrow analysis. Many other authors also used these approaches from different areas **(Hisasue *et al.*, 2001; Wiley *et al.*, 2009; Orazi and Germing, 2009)** for the diagnosis of blood leukemia.
The hematological results that were recorded by **Christina *et al.*, (2017),** including marked to severe leukocytosis due to neutrophilia (90%) with left shift (segmented and band neutrophils), 2% of myeloid blasts, marked non-regenerative anemia, dysplastic platelets, thrombocytopenia, micromegakaryocye and dysplastic neutrophils in a native shepherd dog in Germany. These findings are compatible with the hematological abnormalities of our case. Moreover, Howell-jolly bodies and nucleated RBCs have been observed in the current study during hematological investigations. This might relate to the severity of anemia (RBCs count; 2.6 × 106/ µl, Hb concentration; 3g/dl and Hct; 12%).

A Similar study was made by **Caires *et al.*, (2009)** in Brazil. They reported CML in a five-year-old female Pitbull dog with a severe leukocytosis (122 × 10^3/ µ l) associated with the left shift. They also observed hypercellular bone marrow with a prominent of immature cells and 5.2% myeloid cells.

In 2008, Valli recorded chronic myeloid leukemia with obvious hematological disorders consisting of severe leukocytosis (TLC greater than 50,000 /µl) with marked neutrophilia, left shift, marked thrombocytopenia and non-regenerative. During the histopathological examination, he also found predominant infiltration of neoplastic cells that invaded all internal organs especially, lung, kidney, spleen, and liver **(Valli, 2008).**

Bone marrow analysis by **Christina *et al.*, (2017)** observed a marked hypercellular bone marrow composing of myeloid cells and few erythroid progenitors (1:9 erythroid) myeloid cells ratio), 7% of myeloid blasts, neutrophils lineage and erythroid progenitors dysplasia and a large number of megakaryocytes. Their findings are compatible and have similar characteristics to our case. Another similar work was done by **Joiner *et al.*, (1976).** They observed an extensive amount of reddish-brown bone marrow within the cavity of ribs, femur, sternum, and vertebral bones that refer to hyperplastic bone marrow and enlargement of lymph nodes of internal body organs.

The authors recorded severe leukocyte count (163×10^3/µl, PCV 30%, Hb concentration 9.0g/dl and RBCs count 4×10^6/µl) in the anemic dog with chronic myeloid leukemia. In a study of blood leukemia of a 2-year-old male afghan dog, severe enlargement of the liver (530 g) and spleen (310 g) with slight enlargement of superficial and visceral lymph nodes was recorded **(Holscher *et al.*, 1978)**.

CONCLUSION

This is the first report of chronic myelogenous leukemia in dogs recorded in Iraq. The techniques used in this study provide strong evidence for the diagnosis of the disease. Total white blood cell count (TLC) is usually higher than 100,000 WBC/µl. The number of Myeloid blasts and leukocytes count (neutrophils) in bone marrow is greater than in peripheral blood, as CML starts in pluripotent stem cells of bone marrow. Leukemoid reactions caused by immune-mediated diseases and inflammation should be differentiated from CML. More clinical studies are needed for further investigations and diagnosis such as Cytogenetic Analysis, Flow Cytometry, Computed tomography (CT) scan and Magnetic resonance imaging (MRI) scan.

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Declaration of Conflicting Interests

The authors revealed that there was no potential conflicts of interest.

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