

First Report on Pelger-Huet Anomaly in a Male Basenji Dog in Libya

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Abstract: Pelger-Huet (P-H) anomaly is a benign congenital anomaly of leukocytes, characterized by nuclear hyposegmentation of granulocytes. Patients with heterozygous form of P-H anomaly are not immunodeficient and not predisposed to infection. In this study, P-H anomaly has been detected during a routine blood examination conducted on a clinically normal five years old male Basenji dog. Nuclear hyposegmentation of neutrophils with mature coarse chromatin pattern was noticed. As the animal was in a good health and all other blood parameters were within normal reference range, P-H anomaly was suspected. Acquired, pseudo P-H anomaly was excluded by detecting the same unique nuclear pattern in three successive blood samples collected at month intervals. Nuclear morphology was variable as dumbbell shaped, peanut shaped, band, round or bilobed forms were mostly detected in the neutrophils. It is the first report for this anomaly in Libya.

Key words: Anomaly, Basenji dog, neutrophil, Pelger-Huet

INTRODUCTION

The neutrophil nucleus is not ovoid as in other cell types, but it possesses a lobulated, segmented shape. This deformable nucleus enhances rapid migration (Hoffmann *et al.*, 2007).

In Pelger-Huet anomaly (PHA) which is a hereditary disorder of leukocyte development, nuclear hyposegmentation of granulocytes is a characteristic feature, together with nuclear chromatin hypercondensation (Latimer, 1995).

Recent studies have demonstrated that sufficient cellular levels of a single nuclear envelop integrad membrane protein (Lamin B receptor [LBR]) are necessary for these changes in nuclear architecture (Hoffmann *et al.*, 2007). Abnormalities in sequences of LBR gene result in lack of LBR protein in the nuclear membrane, resulting in hypolobulation and chromatin hypercondensation in the neutrophils of PHA patients (Tomonaga, 2005; Hoffmann *et al.*, 2007; Zwerger *et al.*, 2008).

This anomaly has been reported first in man (Pelger, 1931; Huet, 1932), then in rabbits (Undritz, 1939), dogs (Schalm, 1965; Kiss and Komar, 1967), cats (Weber *et al.*, 1981 and Latimer *et al.*, 1985) and mice (Schultz *et al.*, 2003). This congenital anomaly has not been observed in large animals (Latimer, 2000).

When PHA is encountered in clinical practice, it is usually the heterozygous form. The homozygous form is believed to be lethal in the uterus, and few resulted in stillborn or died within the first months of life

(Latimer, 2000; Latimer *et al.*, 2004). The homozygous form has been rarely observed in humans, cats and rabbits (Oosterwijk *et al.*, 2003; Latimer *et al.*, 1985, 2004). Rare homozygous survived in rabbits and one family of Samoyed dog's exhibit skeletal abnormalities related to chondrodysplasia and ocular problems (Nachtsheim *et al.*, 1950; Aroch *et al.*, 1996). Homozygous PHA has been reported in man without skeletal deformity and may be with prolonged lifespan (Alexieff, 1967; Aznar, 1981; Gastearna *et al.*, 1982).

Advanced biotechnical and cytochemical assays revealed normally functioning leukocytes from PHA affected animals, with no apparent predisposition to infection or immunodeficiency (Latimer and Prasse, 1982; Latimer *et al.*, 1987, 1989; Brockus, 2005).

By itself, heterozygous PHA is not a problem, but it should be differentiated from pseudo-PHA, which is an acquired condition with similar white cell changes. It is mostly associated with other clinical conditions as inflammation and infection, developing leukemia and drug therapy (Latimer, 2000; Tomonaga, 2005). Excluding acquired PHA is necessary to avoid further laboratory tests and/or inappropriate treatment.

In this study heterozygous PHA has been detected in a Basenji male dog, and it is the first report for this anomaly in Libya.

MATERIALS AND METHODS

Clinical case: In January, 2009, a five year-old male Basenji dog was brought for veterinary investigation, as

Table 1: CBC of the dog blood samples

Test	Months				Reference range*
	Jan.	Feb.	Mars	April	
WBC($\times 10^3/\mu\text{L}$)	10.7	11.4	11.3	11.05	5-14.1
Lymphocytes($\times 10^3/\mu\text{L}$)	4.77	2.87	2.85	3.82	0.4-2.9
Monocytes($\times 10^3/\mu\text{L}$)	0.22	0.46	0.46	0.34	0.1-1.4
RBC($\times 10^6/\mu\text{L}$)	7.97	8.49	8.23	8.36	4.95-7.87
Hb(g/dl)	17.8	18.1	17.95	18	11.9-18.9
Hct (%)	57.3	57.1	57.2	57.15	35-57
MCV(fl)	72	67	69.5	68.25	66-77
MCH(pg)	22.3	21.4	21.85	21.6	21-26.2
MCHC(g/dl)	31.0	31.7	31.3	31.5	32-36.3
platelets($\times 10^3/\mu\text{L}$)	415	307	400	410	211-621

*: Latimer *et al.* (2003)

Table 2: Differential count of neutrophils showing the percentage of nuclear types observed

Segmented (tri-lobed)	Bilobed			
	Peanut and dumbbell shaped	Pince-nez form	Band	Metamyelocyte
13	23.5	2.75	58.5	2.25

the owner noticed signs of depression on the animal after it has been left alone with foreigners for few weeks. She requested complete medical checking. Blood sample was collected for Complete Blood Count (CBC).

Lab. Examination: CBC was conducted using automated haematology analyzer (HUMACOUNT-Haematology analyzer - Human GmbH - Gesellschaft für Biochemica und Diagnostica mbH, Germany). Thin blood films were prepared and stained with May-Grunwald-Giemsa stain for evaluation of blood cells morphology and differential neutrophil count; four hundred neutrophils were differentiated and the percentage for each nuclear type is calculated. Blood samples were collected from the dog four times, one month apart. This study was done at the department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Al-Fateh University, Tripoli, Libya.

RESULTS

Clinical examination revealed a normal healthy dog (Fig. 1). Blood parameters obtained were within normal reference range with mild lymphocytosis (Table 1).

On microscopical examination of stained blood films hyposegmentation of neutrophilic nuclei was noticed. Nuclear morphology varied; bands (Fig. 2A, E and H), bilobulated as dumb-bell-shaped (Fig. 2B), peanut-shaped (Fig. 2C) and pince-nez-form (Fig. 2D) were mostly observed. Few metamyelocytes with kidney-shape and oval nuclei (Fig. 2E) and tri-lobed segmenters were occasionally seen. Nuclear chromatin appeared mature and hypercondensed. All neutrophils exhibit normal granularity of cytoplasm.

Eosinophils with round, oval and bi-lobed nuclei were detected with normal cytoplasmic granularity characteristic for this animal species (Fig. 2E and F).



Fig. 1: Male Basenji dog affected with PHA

Monocytes exhibited their normal pleomorphic nuclear morphology (Fig. 2G). Morphological deviations were not noticed in lymphocytes or platelets (Fig. 2H). Haemograms and blood film examination of all blood samples consisted with the previous result (Table 1). A manual differential neutrophil count from February blood sample was conducted to show the percentage of unusual morphological features of neutrophils (Table 2). From blood samples that were collected in the following months, the unique nuclear morphology for the neutrophils persisted and continued to appear, so PHA was confirmed.

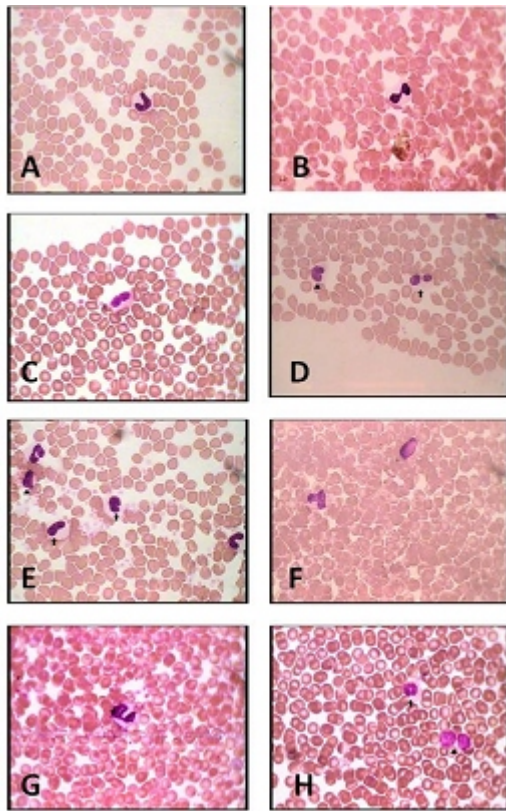


Fig. 2: (A) Bilobed neutrophil with hypercondensed nuclear chromatin. (B) Bilobed neutrophil (dumbbell-shaped) with mature coarse chromatin and normal faint cytoplasmic granules. (C) Bilobed neutrophil with peanut-shaped nucleus and normally appearing platelets. (D) Bilobed neutrophil showing the pince-nez form of nucleus (arrow) and a neutrophilic metamyelocyte with deeply indented nucleus. (E) Two neutrophilic metamyelocytes (arrows), eosinophilic metamyelocyte (arrow head), band neutrophil (right side) and plenty of platelets. (F) Eosinophilic metamyelocyte with oval nucleus containing condensed chromatin, and a nearly band eosinophil with hypercondensed nuclear chromatin. (G) Bilobed monocyte with coarse nuclear chromatin. (H) Band neutrophil with hypercondensed nuclear chromatin (arrow) and two lymphocytes with normal nuclear chromatin condensation (arrow head). *Magnification power x1000*

DISCUSSION

Although automated blood counters supplies the clinician with complete and accurate haemograms, examining a well-prepared and stained blood film remains an excellent choice to get more and may be valuable information concerning blood cells morphology. Many cases of PHA may have been overlooked by missing a

highly informative and nice journey that might have been carried-out with a stained blood film.

In this study, nuclear hyposegmentation resulting from an infection was excluded due to the absence of cytoplasmic toxic changes and the appearance of a fully condensed nuclear chromatin, in contrast, the chromatin pattern of immature, or band neutrophils of infection has a finely granular immature pattern (Latimer *et al.*, 1987; Latimer, 2000).

Furthermore, the morphological differences in PHA cells are persistent, whereas in acquired PHA it is transient and disappears following effective therapy of underlying condition (Latimer, 2000; Logan *et al.*, 2006).

In this case, differential leukocyte count confirm the presence of band, bi-lobed and tri-lobed nuclei with few oval shapes, in homozygous PHA the majority of granulocytes have myelocyte appearance with round to ovoid nuclei containing extremely coarse chromatin pattern (Latimer *et al.*, 1989).

Hyposegmentation in nuclei of eosinophils may not attract the examiner's attention, since nuclear hypolobulation is considered normal in the eosinophils of most animal species (Meinkoth and Clinkenbeard, 2000; Kramer, 2000; Azwai *et al.*, 2007).

In this study the monocytes appeared with variable nuclear morphology as they should be (Meinkoth and Clinkenbeard, 2000). This finding disagrees with the observation of Latimer *et al.* (1987), who documented nuclear hypolobulation of both monocytes and megakaryocytes, and they realized a common stem cell defect in the nuclear lobulation process. However, more recent studies concluded that in other cell lineages as monocytes, lymphocytes and erythroblasts carrying the same defective gene only chromatin-hypercondensation can be observed (Tomonaga, 2005). This morphological appearance was not so obvious in this case, and nuclear chromatin condensation of lymphocytes and monocytes nuclei was almost normal.

The health history of that dog did not detect signs of immunodeficiency or increased susceptibility to infection, this runs parallel with the observations of others who concluded through clinical observations and advanced biotechnical methods normal leukocyte function in PHA affected individuals (Latimer *et al.*, 1987, 1989; Latimer and Prasse, 1982; Brockus, 2005).

CONCLUSION

Although automated blood counters supplies the clinician with complete and accurate haemograms, examining a well-prepared and stained blood film remains an excellent choice to get more and may be valuable information concerning blood cells morphology. Many cases of PHA may have not been diagnosed as a result of

not doing a stained blood film. Research has shown clearly that, although leukocyte morphology is altered, the function of these cells is not affected. Dogs with PHA are at no greater risk for infection than dogs with normal cellular morphology. Both veterinarians and owners should be aware of PHA so that the leukocyte abnormality is not misdiagnosed, leading to further unnecessary diagnostic testing and/or inappropriate treatment.

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