Original Article

Effects of Blood Storage Time and Temperature on Döhle Body or Döhle Body-like Inclusions in Feline Neutrophils

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ABSTRACT

Background: Detecting Döhle body inclusions in cat neutrophils is one of the most relevant toxic changes with clinical significance. It is necessary to study pre-analytical factors such as temperature and blood storage time on the formation of these toxic changes.

Objectives: The present study sought to investigate the impact of blood storage time and temperature on Döhle or Döhle-like inclusions in cat neutrophils.

Methods: Ethylenediaminetetraacetic acid (EDTA) blood samples were obtained from 8 cats without evidence of Döhle inclusions on fresh blood smears (T0). Samples were stored at room temperature (RT) and 4°C as routine storage temperatures of samples in the laboratory. Smears were prepared 2 (T2), 4 (T4), 8 (T8), and 24 (T24) hours following the blood draw for each storage condition. Döhle or Döhle-like inclusions were assessed on each smear randomly selected.

Results: The percentages of neutrophils with Döhle or Döhle-like inclusions in T8 and T24 increased significantly at RT and 4°C, respectively ($P<0.001$) compared to T0. The smears prepared from blood samples stored at RT contained more neutrophils with Döhle or Döhle-like inclusions than 4°C. A significant difference was not found in the percentages of neutrophils with these inclusions between the two temperatures at any storage times.

Conclusion: The development of Döhle body-like in cat neutrophils occurs when the analysis is delayed, especially at higher storage temperatures. This condition may affect diagnosis and clinical decisions. Therefore, the blood smears should be prepared as soon as the blood is drawn to reduce pre-analytical changes.

Keywords: Cat neutrophils, Döhle bodies, Döhle body-like inclusions, Temperature

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1. Introduction

A toxic neutrophil exhibits specific morphologic abnormalities on Romanowsky staining peripheral blood smears. During the maturation process or in association with certain diseases, some changes occur in the bone marrow. The most prominent cytoplasmic changes are Döhle bodies, basophilia, toxic granulation, and vacuolation. Nuclear changes and deviations in cell size and shape can also occur. Döhle bodies are seen in the neutrophils of healthy cats (Gori et al., 2021). In other species, Döhle bodies are seen in animals that exhibit signs of illness and represent toxic change. In neutrophils and their precursors, Döhle bodies are bluish and angular inclusions in the cytoplasm. These structures are retained aggregates of the rough endoplasmic reticulum (Harvey, 2017).

Döhle bodies are more sensitive in human and feline hematology than cytoplasmic vacuolation in diagnosing bacterial infections (Lima et al., 2010). Other researchers found that small and dotted-blue Döhle body-like inclusions are formed due to the accumulation of a rough endoplasmic reticulum or ribosomes and are seen in neutrophils in cats with no clinical signs of inflammation. These components appear to be a false secondary alteration due to prolonged or improper storage of the samples. Since toxic changes in the neutrophils of cats, including Döhle bodies, are clinically significant, it is necessary to know about pre-analytical factors such as storage time and temperature that may cause artificial changes and misdiagnosis of true toxic changes (Aroch et al., 2005). This study aimed to determine the effects of time and temperature on developing Döhle or Döhle-like cytoplasmic inclusions in the neutrophils of clinically healthy cats.

2. Materials and Methods

The study protocol was approved by the local animal Research Ethics Committee at Semnan University of Veterinary Medicine. Informed consent (verbal or written) was obtained from the owner or legal custodian of all animals described in this work for the procedures undertaken.

Eight clinically healthy male cats (DSH and Persian, 4 of each breed with an age range of 2 to 4 years) without any clinical history of anemia and inflammation were selected from cats referred to the Semnan University Veterinary Teaching Hospital (SUVTH) for this study. Two milliliters of blood samples for complete blood count were obtained in potassium EDTA-containing tubes (Non-vacuum K2 EDTA, FAR TEST, IRAN), and examinations were performed within 15 minutes of sample collection (to avoid EDTA storage artifacts) by Celltac α MEK-6500K analyzers (Nihon Kohden) for the use of the most common animal types, like dog, cat, cow, and horse.

One blood smear for differential leukocyte counting and morphologic evaluation was prepared immediately after blood collection and considered baseline time (T0). Three Eppendorf tubes were considered for each temperature, and 150µL of blood was added to each tube. Blood smears were prepared from tubes stored at RT (22°C±3°C) and refrigerator temperature (4°C) after 2, 8, and 24h, and they were stained with Giemsa. There were 56 blood smears (6 blood smears per cat for 2, 8, and 24h and one smear was also prepared for time zero). Each blood smear was evaluated to determine Döhle bodies or Döhle body-like inclusions.

Döhle body-like inclusions are defined as a small dotted, intra-cytoplasmic inclusion of light blue-gray color (Boudrax et al., 2010). Döhle bodies were considered larger intracytoplasmic inclusions, light blue-gray, elliptic to amorphous (Takeuchi et al., 2010) counted as 100 neutrophils per slide, and the percentage of neutrophils with Döhle or Döhle-like cytoplasmic inclusions was recorded.

Statistical analysis was done by SPSS software. First, to normalize the data distribution, their logarithm was calculated. Then the results of the percentage of neutrophils with Döhle body-like inclusions or Döhle bodies between two groups of temperature storage (RT and 4°C) and for each storage period (2, 8, and 24 h) were compared by linear trend repeated measures. The Benjamini-Hochberg method was used to correct the 5% alpha level for multiple pairwise comparisons, and P values less than 0.001 following the correction of the 5% alpha level were considered statistically significant.

3. Results

Neutrophils with Döhle (Figure 1) or Döhle-like cytoplasmic inclusions (Figure 2) were observed in most blood smears. The blood smear examination of all cases, except for cases 1 and 4, prepared from blood stored at RT, showed that the percentage of neutrophils with Dohl or Dohl-like inclusions after 24h was more than 8h (Figure 3). The percentage of neutrophils with Döhle body-like inclusions was higher than Döhle bodies at both temperatures. The percentage of neutrophils

Table 1. Comparing the percentage of neutrophils with Döhle bodies or Döhle body-like inclusions between T2, T8, and T24 with T0 and room temperature (RT) and 4°C

Time \ Temperature	Mean±SD	P	Mean±SD	P	P
	RT		4°C		
T0	5.00±2.15	-	-	-	-
T2	21.00±3.07	0.028[a]	14.00±2.45	0.096[a]	0.129[b]
T8	24.87±4.38	<0.001[a*]	20.37±2.92	0.001[a*]	0.060[b]
T24	41.62±5.39	<0.001[a*]	30.62±3.24	<0.001[a*]	0.111[b]

RT: room temperature; T2: 2h post blood collected, T8: 8h post blood collected, T10: 10h post blood collected.

[a]: Indicated that the comparison between T2, T8, T24, and T0; [b]: Indicated that the comparison between RT and 4°C; *: Indicated significantly different following correction for multiple comparisons.

with Döhle bodies or Döhle body-like inclusions in T8 and T24 increased significantly at RT (P<0.001) and 4°C (P<0.001) compared to T0, respectively (Table 1). The percentage of neutrophils with Döhle or Döhle-like cytoplasmic inclusions in blood samples stored at RT was more than 4°C. No statistically significant difference was observed in the percentage of neutrophils containing Döhle bodies or Döhle body-like inclusions between the two storage temperatures in the T2, T8, and T24 samples (Table 1).

4. Discussion

An integral part of a hematological evaluation is the accurate identification of neutrophil cytoplasmic inclusions. The most common cytoplasmic inclusions in cat neutrophils are Döhle or Döhle-like cytoplasmic inclusions. However, other cytoplasmic inclusions of neutrophils in cats are lysosomal storage granules, including mucopolysaccharidosis VI, VII, and gangliosidosis GM2, red granules in Birman cats (Gough et al., 2018), and other cat breeds, pink-purple granules in Chediak-Higashi syndrome (Bauckley et al., 2020), and large blue thread-like inclusions in May-Hegglin anomaly (Flatland et al., 2011). False detection of leukocyte inclusions is associated with serious clinical consequences, including misdiagnosis, incorrect treatment, and poor prognosis.

The manifestation of Döhle or Döhle-like cytoplasmic inclusions in cat neutrophils in the present study (Figure 1) increased after 8h of blood sampling relative to baseline time at both room and refrigerator temperatures. This increase with a steeper slope in most samples kept at room temperature, except for the first and fourth samples, which reached their highest percentage in 24h. While at refrigerator temperature, the slope of increasing the percentage of neutrophils with Döhle-like bodies or

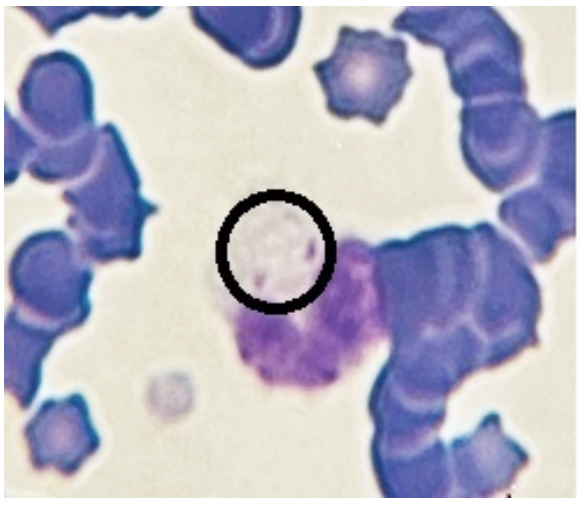

Figure 1. Döhle bodies circled in black in cat neutrophil

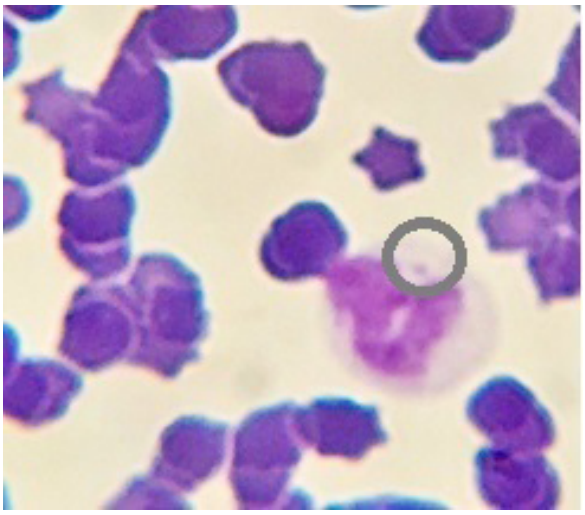

Figure 2. Döhle body-like inclusion circled in gray in cat neutrophil

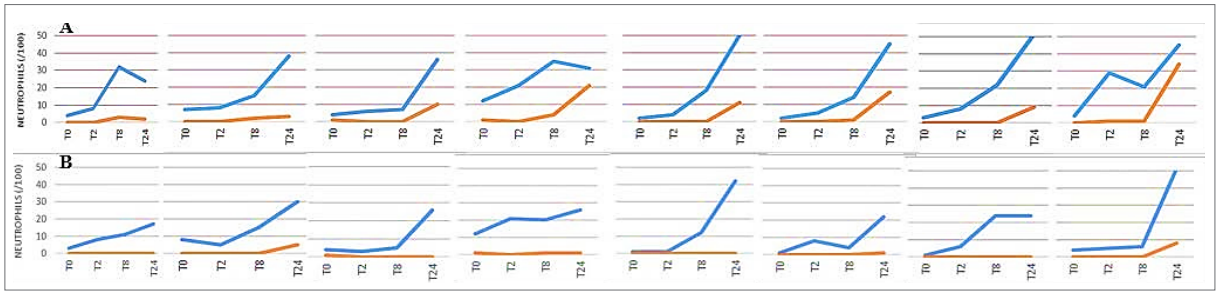

Figure 3. Histogram showing the progressive appearance of Döhle or Döhle-like cytoplasmic inclusions within each case for each temperature and time point

The Döhle-like cytoplasmic inclusions are displayed in orange, and the Döhle bodies are displayed in blue.

A: Room temperature; B: 4°C; T2: 2h post blood collected; T8: 8h post blood collected; T24: 24h post blood collected.

inclusions was milder. It must be mentioned that the increase in the percentage of neutrophils with Döhle-bodies or Döhle body-like inclusions at room temperature was more significant than the refrigerator temperature. This finding was consistent with the results of a study by Bau-Gaudreault et al. (2019), which examined the impact of duration and maintenance on toxic or semi-toxic changes in neutrophils of dogs. According to Table 1, there was no statistically significant difference between the RT and refrigerator temperatures in the percentage of neutrophils with Döhle or Döhle-like cytoplasmic inclusions during storage times of 2, 8, and 24h.

Since the actual toxic change in bone marrow neutrophils occurs before diffusion into the peripheral blood (Takeuchi et al., 2010), the nature of the Döhle body-like inclusions observed in the present study is uncertain. The inclusions are thought to result from accumulated rough endoplasmic reticulum or ribosomes over time, in which case the cells become more permeable to staining, either due to the staining of previously invisible organs or the destruction of existing organs. Evaluation of these smears by electron microscopy can significantly help clarify their origin. Blood smears assessment to evaluate toxic neutrophil changes is a cheap, fast, simple, and accessible process that indicates the infectious and metabolic diseases in cats. Observation of toxic neutrophils is a significant diagnostic finding and an aid in patient evaluation, disease course, length of hospital stay, and treatment planning. In cats, unlike dogs, toxic neutrophils are not associated with higher mortality (Gori et al., 2021). Various reports have shown time- and temperature-dependent changes in erythrocytes, white blood cells, platelets, and automatic and manual CBC markers in cattle (Ihedioha et al., 2007), laboratory animals, dogs, sheep, goats, horses, turkeys, and sea lions (Hadzimusic et al., 2010). The time delay between sampling and analysis is when blood samples are sent to reference laboratories or when the analysis cannot be performed easily, affecting the quality of the analysis. Ameri et al. (2011) found that although most changes in the blood tests of monkeys, rabbits, mice, and rats were clinically insignificant when the sample was stored at 4°C, the best way to test the blood of these animals is to process the blood promptly, preferably 1 hour after blood collection. In the present study, no other toxic changes, such as basophilic cytoplasm and foamy vacuolation, were observed in feline neutrophils. Perez-Ecija et al. (2020) found that increased basophilia and foamy vacuolation of the neutrophil cytoplasm in the smears within 1 hour of blood collection indicated inflammatory disease in donkey blood. Regarding the slight increase in foamy vacuolation that occurs over time in EDTA, the prominence of moderately vacuolated neutrophils in the smears that occur a few hours after blood collection is questionable. Though, moderate or severe foamy vacuolation should be considered clinically.

5. Conclusions

Based on the current study, morphological changes in neutrophils of healthy cats developed in vitro in addition to Döhle or Döhle-like cytoplasmic inclusions, including low foamy vacuolation, without cytoplasm basophilia, which were different from neutrophils are associated with severe inflammation. Therefore, determining and interpreting toxic changes in cat neutrophils are affected by storage conditions and time. Hence, it is suggested that a freshly prepared blood sample be sent to the laboratory immediately after blood sampling with a blood sample stored at 4°C. It is also recommended that the time and date of the blood sampling be clearly stated on the submitted samples.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Semnan University of Iran (Code: E-98/09).

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Authors' contributions

Conceptualization and Supervision: Mahmood Ahmadi-hamedani; Methodology: Mahmood Ahmadi-hamedani; Investigation, Writing-original draft, and Writing-review & editing: All authors; Data collection: Mahmood Reza Tabrizchi; Data analysis: Mahmood Ahmadi-hamedani; Funding acquisition and Resources: Mahmood Ahmadi-hamedan and Mahmood Reza Tabrizchi.

Conflict of interest

The authors declared no conflicts of interest.

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References

Ameri, M., Schnaars, H. A., Sibley, J. R., & Honor, D. J. (2011). Stability of hematologic analytes in monkey, rabbit, rat, and mouse blood stored at 4 C in EDTA using the ADVIA 120 hematology analyzer. *Veterinary Clinical Pathology, 40*(2), 188-193. [DOI:10.1111/j.1939-165X.2011.00304.x] [PMID]

Aroch, I., Klement, E., & Segev, G. (2005). Clinical, biochemical, and hematological characteristics, disease prevalence, and prognosis of dogs presenting with neutrophil cytoplasmic toxicity. *Journal of Veterinary Internal Medicine, 19*(1), 64–73. [DOI:10.1111/j.1939-1676.2005.tb02660.x] [PMID]

Bau-Gaudreault, L., & Grimes, C. N. (2019). Effect of time and storage on toxic or pseudo-toxic change in canine neutrophils. *Veterinary Clinical Pathology, 48*(3), 400-405. [DOI:10.1111/vcp.12755] [PMID]

Boudreaux, M. K., Osborne, C. D., Herre, A. C., Rivera, E. R., & Spangler, E. A. (2010). Unique structure of the M loop region of β1-tubulin may contribute to size variability of platelets in the family Felidae. *Veterinary Clinical Pathology, 39*(4), 417-423. [DOI:10.1111/j.1939-165X.2010.00256.x] [PMID]

Buckley, R. M., Grahn, R. A., Gandolfi, B., Herrick, J. R., Kittleson, M. D., & Bateman, H. L., et al. (2020). Assisted reproduction mediated resurrection of a feline model for Chediak-Higashi syndrome caused by a large duplication in LYST. *Scientific Reports, 10*(1), 64. [PMID]

Flatland, B., Fry, M. M., Baek, S. J., Bahn, J. H., LeBlanc, C. J., & Dunlap, J. R., et al. (2011). May-Hegglin anomaly in a dog. *Veterinary Clinical Pathology, 40*(2), 207-214. [DOI:10.1111/j.1939-165X.2011.00320.x] [PMID]

Gori, E., Pierini, A., Lippi, I., Lubas, G., & Marchetti, V. (2021). Leukocytes ratios in feline systemic inflammatory response syndrome and sepsis: A retrospective analysis of 209 cases. *Animals : An Open Access Journal from MDPI, 11*(6), 1644. [DOI:10.3390/ani11061644] [PMID] [PMCID]

Gough, A., Thomas, A., & O'Neill, D. (2018). *Breed predispositions to disease in dogs and cats*. New Jersey: John Wiley & Sons. [DOI:10.1002/9781119225584]

Hadzimusic, N., Katica, M., Muharemovic, Z., & Muanovic, J. (2010). Effect of temperature storage on hematological parameters of avian turkey blood. *International Journal of Collaborative Research on Internal Medicine & Public Health, 2*(5), 158-166. [Link]

Harvey, J. W. (2017). The feline blood film: 2. Leukocyte and platelet morphology. *Journal of Feline Medicine and Surgery, 19*(7), 747-757. [DOI:10.1177/1098612X17706471] [PMID]

Ihedioha, J. I., & Onwubuche, R. C. (2007). Artifactual changes in PCV, hemoglobin concentration, and cell counts in bovine, caprine, and porcine blood stored at room and refrigerator temperatures. *Veterinary Clinical Pathology, 36*(1), 60-63. [DOI:10.1111/j.1939-165x.2007.tb00183.x]

Lima, M. L. F., Soares, P. T., Ramos, C. A. N., Araújo, F. R., Ramos, R. A. N., & Souza, I. I. F., et al. (2010). Molecular detection of Anaplasma platys in a naturally-infected cat in Brazil. *Brazilian Journal of Microbiology, 41*(2), 381-385. [DOI:10.1590/S1517-83822010000200019]

Perez-Ecija, A., Buzon-Cuevas, A., Aguilera-Aguilera, R., Gonzalez-De Cara, C. A., & Mendoza, F. J. (2020). Blood storage conditions affect hematological analysis in samples from healthy donkeys and donkeys with experimentally-induced endotoxemia. *Frontiers in Veterinary Science, 7*, 640. [DOI:10.3389/fvets.2020.00640] [PMID] [PMCID]

Takeuchi, Y., Iizuka, H., Kanemitsu, H., Fujino, Y., Nakashima, K., & Uchida, K., et al. (2010). Myeloma-related disorder with leukaemic progression in a cat. *Journal of Feline Medicine and Surgery, 12*(12), 982-987. [DOI:10.1016/j.jfms.2010.07.018] [PMID] [PMCID]

مقاله پژوهشی

اثرات دما و زمان نگهداری خون بر گنجیدگی های دل و شبه دل در نوتروفیل های گربه

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چکیده

زمینه مطالعه: اهمیت بالینی تشخیص گنجیدگی‌های دل در نوتروفیل‌های گربه به عنوان یکی از مرسوم ترین تغییرات توکسیک، مطالعه عوامل پیش تحلیلی مانند دما و زمان ذخیره سازی خون در شکل‌گیری این تغییرات را ضروری کرده است.

هدف: مطالعه حاضر به دنبال بررسی تأثیر زمان و دمای ذخیره سازی خون بر گنجیدگی‌های دل یا شبه دل در نوتروفیل های گربه بود.

روش کار: نمونه خون EDTA دار از هشت گربه بدون شواهدی از گنجیدگی های دل در گسترش های خون تازه (T0) بدست آمد. نمونه‌ها در درجه حرارت اتاق (RT) و ۴ درجه سانتی‌گراد، به عنوان دماهای متداول نگهداری نمونه‌ها در آزمایشگاه نگهداری شدند. گسترش ها ۲ ساعت (T2)، ۸ ساعت (T8) و ۲۴ ساعت (T24) پس از خونگیری برای هر دو دمای ذخیره سازی تهیه شدند. گنجیدگی های دل یا شبه دل در هر گسترش به صورت رندوم ارزیابی شدند.

نتایج: درصد نوتروفیل های دارای گنجیدگی های دل یا شبه دل در T8 و T24 به ترتیب در درجه حرارت اتاق و ۴ درجه سانتی‌گراد در مقایسه با T0 به طور معنی‌داری افزایش نشان داد ($P<0/001$). گسترش های خونی تهیه شده از نمونه های خون نگهداری شده در درجه حرارت اتاق دارای نوتروفیل‌های حاوی اجسام دل یا گنجیدگی های شبه دل بیشتری نسبت به نمونه‌های خون ذخیره شده ادر ۴ درجه سانتی‌گراد بود. اختلاف آماری معنی داری در درصد نوتروفیل های دارای این گنجیدگی‌ها بین دو دما در هیچ یک از زمان های ذخیره سازی یافت نشد.

نتیجه‌گیری نهایی: ایجاد گنجیدگی های شبه دل در نوتروفیل‌های گربه زمانی اتفاق می‌افتد که آرمایش خون به تاخیر بیفتد، به خصوص در دمای ذخیره‌سازی بالاتر، و ممکن است بر تشخیص و تصمیم‌گیری بالینی تأثیر بگذارد. بنابراین، یک گسترش خون باید به محض خون‌گیری تهیه شود تا تغییرات پیش از آزمایش کاهش یابد.

کلیدواژه‌ها: نوتروفیل گربه، اجسام دل، گنجیدگی های شبه دل، دما، زمان

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