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MORPHOLOGICAL STUDY OF RED BLOOD CELLS AS A TOOL FOR ANIMAL SPECIES DENTIFICATION

(With 2 Tables, 2 Plates and 2 Figures)

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دراسة مورفولوجية لكرات الدم الحمراء كوسيلة للأستعراف على الفصائل الحيوانية

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أجريت هذه الدراسة لتقييم استخدام بعض القياسات المورفولوجية لكـــرات الـدم الحمـراء لبعض الفصائل الحيوانية المختلفة كوسيلة للاستعراف عليها. تم تحضير مسحات دموية لـدم جمع حديثا من فصائل مختلفة (ثدييات، طيور، أسماك ، برمائيات) وقد تم تحضـــير عشـر مسحات دموية من عشر حيوانات لكل فصيلة. وتم صباغتها بصبغة الجمسا واجراء الفحص و القياسات على عشرين خلية من كل مسحة بواسطة برنامج كمبيوتر متخصص. حيث تـــم قياس كل من القطر والمساحة الكلية والمحيط لكل خلية. كما تم قياس المساحة الكلية للنـــواه فى الخلايا المحتوية عليها ومقارنتها بالمساحة الكلية لها. وقد أظهرت النتائج وجود اختـلاف حاد فى قياس محيط كرات الدم بالنسبة للعينات موضع الدراسة حيث وجد أن قياس المحيـط هو ٤,٥ ، ٥,٢ ، ٥,٧ ، ٥,٩ ، ٦,٠ ، ٧,٠ ، ٧,٥ ، ٧.٧ ، ٧,٤ × ٤,٣ ميكـــرون فى كل من الأغنام والأبقار والجاموس والقطط والحمـــير والكـــلاب والارانـــب والأنســـان والجمال على التوالي. وأن أصغر مساحة لسطح خلايا الدم الحمراء وكذلك محيطـــها فـــي كرات دم الأغنام هي ١٦,٢ ميكرون مربع و١٥,٧ ميكرون على التوالى، والتى تمتلك كذلك أصغر قطر وهو ٤,٥ ميكرون. بينما لوحظ أن أكبر مساحة سطح ومحيط فـــى كـــرات دم الأنسان حيث وصلت الى ٤٢.٣٤ ميكرون مربع و٢٦,٤ ميكرون على التوالى) والتى لـــها أكبر قطر (٧.٧ ميكرون). بينما فى الفصائل المحتوية على نواه وجد أن أكبر قطر و محيط ومساحة سطح قد سجلت فى الضفادع لتصـــل الـــي ٢٣,٣ x ١٤,٧ و ٦٧,٩ ميكـــرون و ١١٠,٩ ميكرون مربع على الترتيب. وأقل قطر و محيط ومساحة سطح قـــد ســـجلت فـــى أسماك القراميط لتصل الي ٩,٨ x ٧,٢ و ٣١,٥ ميكرون و ٥٦,٩ ميكرون مربع علـــى الترتيب. وبمقارنة مساحة سطح النواه بالنسبة للمساحة الكلية وجد أنها تأخذ نســـبة ١٧,٥ ، ٢,٨ ، ٢٥,٥ ، ٢٤,٠٥ ، ١٩,٠، ٢٢,٦ ، ٢٤,٣، ١٦,٠، ٢٣,٤ % بالنســـبة للدواجـــن والرومى والبط والأوز والحمام والضفادع والبلطى والقرموط والمبروك على الترتيب. ومن النتائج السابقة يتضح أنه يمكن الأستعراف على الحيوان من خلال فحص البقعـــة الدمويـــة

ومقارنة قطر ومساحة السطح والمحيط بالأنواع الأخرى. وتستخدم نفس الطريقـــة بالنســبة لكرات الدم المحتوية على نواه بالإضافة الى معرفة نسبة مساحة النواة للمساحة الكلية. وعلى ذلك فهي تعتبر وسيلة جيدة للأستعراف خاصة بالنسبة لكرات الدم المحتوية على نواه والتـى لم يسبق ايجاد وسيلة للتفرقة بينها.

SUMMARY

The present study recorded the different morphological measurements of red blood cells in different mammals, birds, fish, and amphibian as a tool for identification. Blood smears were prepared from fresh-collected blood of individual species (mammals, birds, fish and amphibian). Ten blood samples were collected from each investigated species. The blood smears were stained with Geimsa stain. Twenty cells from each sample were examined and measured by the computerized program. Diameter, total surface area and contour of each cell were measured. In addition the total surface area of the nucleus, in nucleated cell was measured and the ratio between it and total area was calculated. Our results revealed that, the cell diameter was 4.5, 5.2, 5.7, 5.9, 6.0, 7.0, 7.5, 7.7 and 7.4 x 4.3 µm in sheep, cow, buffalo, cat, donkey, dog, rabbit, human and camel respectively. The smallest area and contour were recorded in sheep (16.2 ± 2.3 μm^2 and 15.7 ± 1.5 µm), where it has also the smallest diameter (4.5 ± 0.5 µm). The largest surface area and contour were recorded in human red blood cells (42.3 ± 5.4 μm^2 and 26.4 ± 2.5µm) which have the largest diameter (7.7 ± 0.6 µm). In nucleated red blood cells, the maximum diameter, contour and surface area were recorded in frog (23.2 x 14.7, 67.9 ± 3.0 µm and 110.9 ± 2.5 μm^2). The minimal diameter, contour and surface area were recorded in Clarias gariepinus fish, (9.8 x 7.2, 31.5 ± 2.6 µm and 56.9 ± 8.5 μm^2). The results indicated that the ratio of nucleus surface area to the total surface area (%) of the cell was 17.5, 20.8, 25.5, 24.05, 19.0, 22.6, 24.3, 16.0 and 23.4 % of the total area in chicken, turkey, duck, Goose, Pigeon, Frog, Oreochromis niloticus, Clarias gariepinus and Ctenopharyngodon idella respectively.

***Key Words**: Red blood cells – mammals – bird – fish – amphibian.*

INTRODUCTION

Blood stains are of great importance as a criminal evidence in forensic medicine field. Examination of blood stains is a fundamental step helps in detection of diagnostic information (exposure to diseases or toxicants) and species determination.

Red blood cells, the most numerous cells in blood are non-nucleated in mammals (Richard et al., 1993) and nucleated in birds, fish and amphibian (Gradwahl, 1956).

Results of red blood cell diameter measurements recorded by Duckes (1955), Smith and Fiddes (1955), Soliman (1966), Breazile (1971) Shehata and Ibrahim (1984), and Jain (1993) showed great individual variation between mammals. No available information was recorded about variable measurements in species having nucleated red blood cells (Birds, Fish and amphibians).

Normal mammalian erythrocytes are generally biconcave disks with a light center and a small rim of hemoglobin. A slight variation in size is common among erythrocytes of various species. A variation in shape may be artifactual (such as crenation), a natural occurrence (sickle cells in the deer and fusiform and spindle-shaped red cells in Angora goats), or a pathologic abnormality. Rouleau formation (rolls of red blood cells, similar to a pile of coins) is prominent in the horse, and some rouleau is apparent in dog and cat blood. Increase rouleau formation in the dog and cat suggests an increase in total plasma protein concentration, globulin, or fibrinogen. Bovine erythrocytes normally do not form roulcaux. The erythrocytes of family Camelidae includes camels, guanacos, llamas, alpacas, and vicunas, are elliptic compared to reticulocytes and nucleated red cells, which appear to be round, the red cells lack a central depression and are thin (Jain, 1993).

Normal canine RBCs are biconcave with an area of central pallor occupying about one third of the cell's diameter. Feline RBCs do not consistently have discernible central pallor and tend to vary slightly more in shape than canine RBCs. Both species have mild RBC anisocytosis and an occasional immature polychromatophilic cell in peripheral circulation, Cowell et al. (1998).

The estimation of red blood cell diameter previously recorded by Duckes (1955), Smith and Fiddes (1955) Soliman (1966), Breazile (1971) Shehata and Ibrahim (1984), and Jain (1993), showed a limited value in the detection of species identification.

Cytological investigation of red blood cell through examination of blood smear may be a useful tool for medicolegal practitioner in determining animal species. The use of either precipitin test or chromosomal investigation in the field of animal identification is complicated and very expensive. Therefore identification through morphological measurements of red cells has several advantages, where its preparation is easy and inexpensive. Also blood smear examination is readily learned with adequate background information and routine practice. Concerning the absence of data evaluating (recording) red cell measurements, other than diameter in non-nucleated type, in the present work we tried to investigate some red cell measurements as diameter, total surface area, and contour in non-nucleated cells and length, width, total surface area, area of cytoplasm, area of nucleus and contour in nucleated cells. We hope to establish a simple easy and rapid method for animal species identification.

MATERIALS and METHODS

In the present study nine species of mammals (Sheep, Cow, Buffalo, Cat, Donkey, Dog, Rabbits, Human and Camel), five species of birds (Chicken, Turkey, Duck, Goose, and Pigeon), three species of fish (Oreochromis niloticus {Nile tilapia}, Clarias gariepinus {Sharptoothcatfish} and Ctenopharyngodon idella {Grass carp}) and one amphibian (frog) were investigated.

Ten blood samples were collected from each species in clean tubes containing anti-coagulant substance (EDTA). Blood films from the obtained samples were prepared and fixed by methyl alcohol and stained with Geimsa stain. Twenty cells in each film were examined microscopically for estimation of diameters, total surface area, contour and total surface area of the nucleus (in nucleated cells). Cell measurements were obtained by the main computerized program for measurements (Image analysis system, Leica Q500). The relation between surface area of the nucleus and the total surface area of the cell and also determination of total surface area of the cytoplasm were calculated from the obtained results.

Student's t-test was used to determine the statistical analysis (Gad and Weil, 1986).

RESULTS

Results obtained were summarized in Tables 1 and 2, also the morphological differences were recorded in Plates 1 and 2.

Morphological examination and measurements revealed that, the largest non-nucleated red blood cells diameter was recorded in human (7.7 µm) and the smallest one was recorded in sheep (4.5 µm). In nucleated red blood cells, the largest cell (length x width) was recorded in frog (23.2 x 14.7 µm) while the smallest cell was recorded in Clarias gariepinus fish (9.8 x 7.2 µm). The largest nucleus area measurement was recorded in frog (25.1 μm^2) and the smallest nucleus was recorded in also in Clarias gariepinus fish (9.1 μm^2). While the largest nucleus area compared to the total surface area of the cell was recorded in duck which takes about 25.5% of total surface area, while the smallest nucleus area was recorded in Clarias gariepinus fish, which takes about 16% of total surface area.

DISCUSION

The red blood cells have special features in each animal. In dog its uniform in size. There is occasional Heinz bodies in cat, giant forms in cow and regular size and shape with small central pale spot in sheep (Jain, 1993). Same observation was recorded in our study (Plate 1). He also recorded that the RBCs diameters in different animals were 4.5, 5.8, 5.8, 6.2, 6.7, 7.0 and 7.5 x 4.4 µm in sheep, cow, cat, donkey, rabbit, dog and camel respectively. These informations are quite similar to our results in non-nucleated red blood cells. The same author mentioned that avian erythrocytes are generally elliptic and large. The nucleus also elliptic and has condensed chromatin. The cytoplasm appears orange-pink.

Shehata and Ibrahim (1984) recorded that the RBCs diameters in cat, dog, rabbit, sheep, goat, cow, ass., horse, human and buffalo were 5.85, 7.7, 6.96, 3.65, 3.0, 4.84, 5.84, 5.3, 7.6 and 5.5 µm respectively.

Smith and Fiddes (1955) also recorded that the cell diameters were 7.5, 6.0, 4.0, 2.0, 22 x 15 and 14 x 6 µm in human, cat, goat, deer, frog and pigeon respectively.

Erythrocytes of human may be described as biconcave discs, they are thicker at the edges than in the middle. The average red blood cell is about 7.5 µm in diameter and about 2 µm thick at the edges. The essential role of erythrocytes is to transport oxygen to the respiring

tissues and carry carbon dioxide away from the tissues. Red blood cells are ideally designed both in form and content for these tasks (Haen, 1995).

Cowell et al. (1998) mentioned that normal canine (dog) RBCs are 7 µm in diameter, slightly larger than the 5.5 – 6.5 µm diameter of feline RBCs, that results agree with ours where dog's RBCs diameter about 7 µm and cat about 5.9 µm.

We can conclude that there is species variance indicated by measuring the diameter, surface area, and contour. As well as the ratio between surface area of the nucleus and the total surface area in the nucleated red blood cells. So the morphometric measurements were recorded as a good method to differentiate between different animal species specially the nucleated cells species (bird, fish and amphibian) as well as their species.

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Table 1: Measurements (mean ± S.D.) of non-nucleated red blood cells

Parameters	Diameter (μm)	Total surface area (μm^2)	Contour (μm)
Sheep	4.5 ± 0.5	16.2 ± 2.3	15.7 ± 1.5
Cow	5.2 ± 0.4	20.2 ± 1.9	16.9 ± 1.1
Buffalo	5.7± 0.6	22.7 ± 3.1	19.4 ± 1.5
Cat	5.9 ± 0.5	25.0 ± 3.5	20.0 ± 1.5
Donkey	6.0 ± 0.6	27.5 ± 3.7	20.8 ± 1.9
Dog	7.0 ± 0.9	32.6 ± 3.7	21.6 ± 1.9
Rabbit	7.5 ± 1.4	36.0 ± 6.7	23.7 ± 3.2
Human	7.7 ± 0.6	42.3 ± 5.4	26.4 ± 2.5
Camel (length x width)	7.4 ± 0.8 x 4.3 ± 0.6	24.6 ± 3.6	20.4 ± 1.9

Table 2: Measurements (mean ± S.D.) of nucleated red blood cell

Parameters	Length (μm)	Width (μm)	Surface area (μm^2)	Area of cytoplasm (μm^2)	Area of nucleus (μm^2)	Contour (μm)	% of nucleus
Chicken	12.1 ± 0.8	6.9 ± 0.7	66.2 ± 8.0	54.6 ± 10.4	11.6 ± 1.9	35.5 ± 2.6	17.5
Turkey	12.6 ± 0.9	7.8 ± 0.8	83.02 ± 10.1	65.7 ± 11.2	17.3 ± 3.4	37.2 ± 2.0	20.8
Duck	13.9 ± 1.1	8.2 ± 0.7	89.3 ± 10.9	66.5 ± 12.4	22.8 ± 3.2	40.7 ± 3.5	25.5
Goose	15.3 ± 0.5	8.3 ± 0.3	95.2 ±8.1	72.3 ± 9.4	22.9 ± 2.2	44.2 ± 3.1	24.05
Pigeon	12.2 ± 1.1	7.2 ± 0.8	69.7 ± 12.2	56.4 ± 12.3	13.3 ± 3.0	35.1 ± 3.0	19.0
Frog	23.2 ± 1.3	14.7 ± 0.5	110.9 ± 10.2	85.9 ± 9.3	25.1 ± 2.0	67.9 ± 3.0	22.6
Oreochromis niloticus	11.5 ± 1.5	8.6 ± 1.4	72.9 ± 12.4	55.2 ± 14.6	17.7 ± 2.8	35.9 ± 4.7	24.3
Clarias Gariepinus	9.8 ± 1.0	7.2 ± 0.8	56.9 ± 8.5	47.8 ± 8.5	9.1 ± 2.9	31.5 ± 2.6	16.0
Ctenophary-ngodon idella	12.2 ± 0.6	7.3 ± 0.7	69.7 ± 7.5	53.4 ± 5.1	16.3 ± 3.0	35.4 ± 2.2	23.4

Fig. 1: Relationship of diameter to total surface area of the non-nucleated red blood cells by µm and µm².

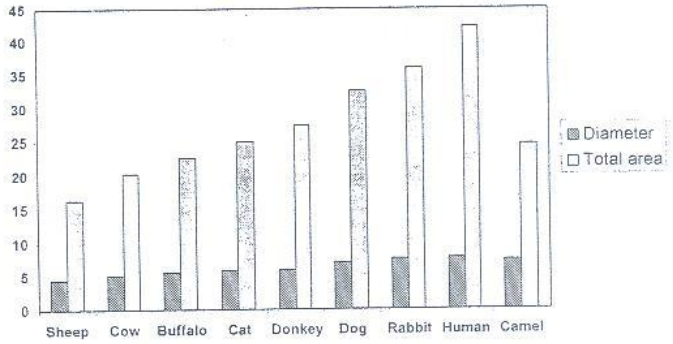
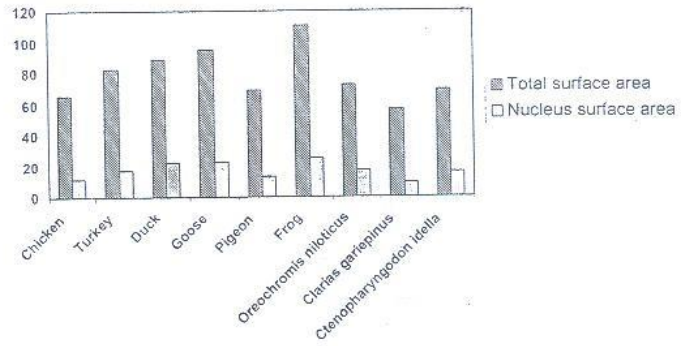


Fig. 2: Relationship of total surface area to surface area of the nucleus in nucleated red blood cells by µm².



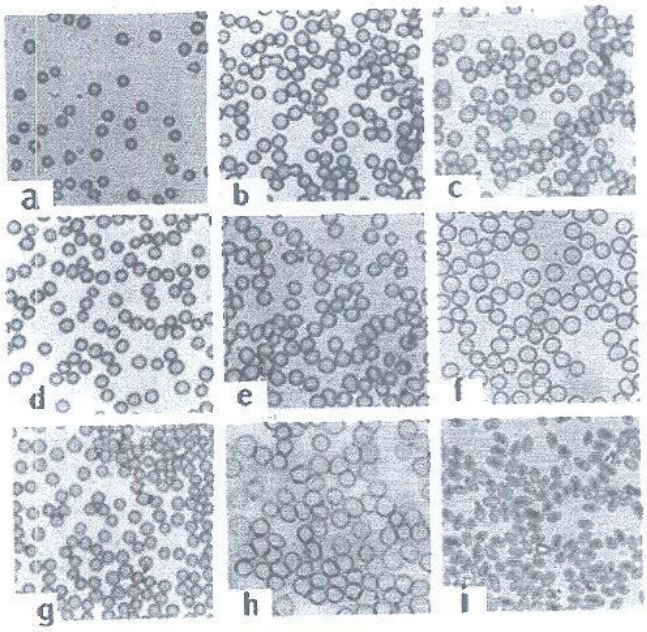


Plate 1: Non-nucleated red blood cells **(a)** Sheep, **(b)** Cow, **(c)** Buffalo, **(d)** Cat, **(e)** Donkey, **(f)** Dog, **(g)** Rabbit, **(h)** human, **(i)** Camel. Stained with Geimsa stain, X 40.

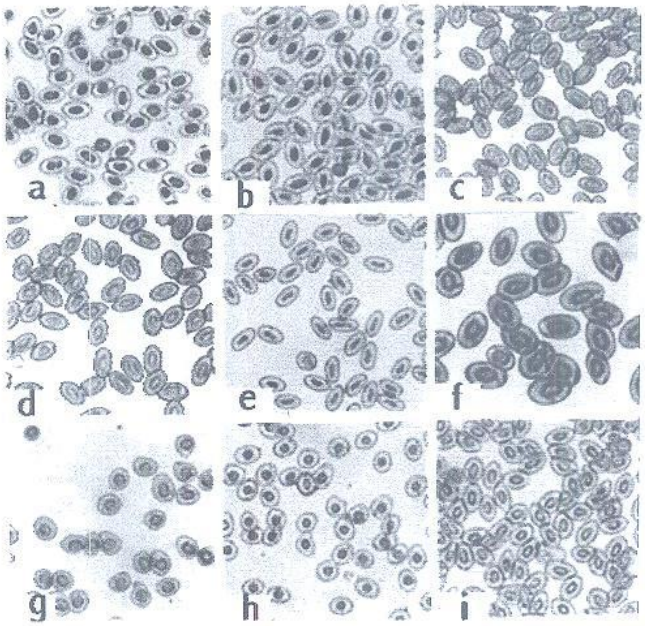


Plate 2: Nucleated red blood cells **(a)** Chicken, **(b)** Turkey, **(c)** Duck, **(d)** Goose, **(e)** Pigeon, **(f)** Frog, **(g)** Oreochromis niloticus, **(h)** Clarias Gariepinus, **(i)** Ctenopharyngodon idella. Stained with Geimsa stain, X 40.