A comparative histological study on the blood film of young and adult, male and female Monkeys (*Chlorocebus pygerythrus*)

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Summary

Twenty Vervets monkeys were used to study the effect of sexes and ages on morphohistological and hematological pictures. The study revealed that age and sex affect blood values. Vervet monkeys registered a significant dominancy of hematological parameters in adult males. Erythrocytes of vervet monkeys were of macrocytic type, howell-Jolly bodies were observed. Rouleaux formation, Anisocytosis, Aggregation, and Agglutination were not observed. A significant predominancy of neutrophils in females and lymphocytes in males was recorded. The neutrophil was well defined, large with ring-shaped highly segmented nucleus. Band neutrophils were not observed in peripheral blood. Eosinophils recorded high values. Circulating leukocytes acted as a transient phase in the functional development of tissue leukocytes. Lymphocytes were the most common cells among leukocytes. Three sizes of lymphocytes and two of monocytes were registered. The value of monocytes percent was higher in males. The herein study declared that blood platelets showing different sizes, shapes and affinities for basic stains and according to these functional affinity, light resting and dark stimulating blood platelets were classified. There was a reverse relationship between the number of erythrocytes and blood platelets among different sexes and ages. In conclusion, the present study hypothesized that blood platelets serve as a supporting cells for blood cells especially erythrocytes. Besides, humoral immune defense in monkeys were well developed.

Keywords: Blood film, Male monkey, Female monkey, Histology.

Introduction

Non-human primates play an important role in research into human disease because of their close genetic relationship to humans. Many previous studies have reported a hematological data in Cynomolgus, Rhesus, and Vervet monkeys caught in the wild (1). The vervet monkeys have been noted for having-human like characteristics, such as hypertension, anxiety, and social dependent alcohol use (2). Not all investigators have taken the influences of such factors as sex, age, altitude, nutrition and length of captivity into account (3). So that the present study found to choose the vervet monkeys as a model to understanding the uniform blood picture. Many studies on hematological values of primate species were reported in many literatures (4), however most of these studies were done on the genus (Macaca mulatta) (5), and (Cebus apella) (6 and 4). Sasseville et al. (7) reported that erythrocytes were subjected to variation that depends on sex, age, habitat, and length of captivity. Erythrocyte in human, monkey and dog were of macrocyte type, reversely in goat as of microcyte (8). RBCs count, Hb, PCV and WBCs count were higher in males than in females (7 and 9). Immature granulocytes (band cell), normally absent from peripheral blood (6 and 10). Neutrophil was the predominant leukocyte in non-human primates (11). Lymphocytes are the predominant leukocytes in ruminants and pigs (12). It is difficult to differentiate between monocyte and large lymphocyte (13). Platelets are very different in size, metabolism, and functional activity. The diameter varies with species with cat having larger platelets than other domestic animals (13 and 14). Newly released platelets are more reactive than mature platelets. Newly formed platelets have higher RNA content (15). The aim of this study was to investigate the effect of sexes and ages on morphohisto-logical and hematological pictures.

Materials and Methods

Twenty apparently healthy young and adult, males and females vervet monkeys were prepared. The animals were divided into four groups according to sex and age (5 for each).

The young males weighed 1075 to 3100 gm, the adult males weighed 3950 to 5090 gm, the young females weighed 1015 to 2100 gm, and the adult females weighed 3350 to 4025 gm. The age of young animals range between 8 months to 2 years. The age of adult animals range between 2.5 to 6 years. All animals were housed indoor in Baghdad at spring season. The temperature was 10-26°C with relative humidity of 31%-52%. To avoid fighting, the animals were kept together as one adult male was put with other young and adult females. The drawing of blood was done collectively to get rid of anxiety (2). The femoral vein was used for withdrawal of blood (with and without K2-EDTA anticoagulants) from non-anesthetized monkeys. The blood smears were fixed with methanol and stained by modified wright and giemsa stains and higher magnification of histological examination were performed (16). Complete blood counts including (RBCs, Hb, PCV, WBCs, differential leukocytic, and blood platelets) were done (17). Statistical analysis of data was carried out on the basis of two ways: (ANOVA and LSD) (18).

Results and Discussion

The present study revealed that sex and age of vervet monkeys affect the values of blood. Adult males recorded higher values of RBCs count, Hb, PCV, MCV, MCH, MCHC, and WBCs count than other groups with the exception of platelets count which was higher in young females (Tables, 1). The present study registered that the erythrocytes of vervet monkey were of macrocytic type. The central pallor occupied more than one third of the cell (hypochromia) (Fig. 1). This result was similar to that found in (Macaca mulatta) (4), and dog (19), and different from cat, cattle, and horse (13). Vervet monkeys did not exhibit rouleaux formation, anisocytosis, aggregation and agglutination that differ from dog, cat, horse, and rarely from ruminants and pig (12 and 13). Howel-Jolly bodies were observed (Fig. 2). This finding was similar to that found in horse and cat (13).

Lymphocytes were the most frequently observed leukocytes in peripheral blood, followed by neutrophils (Table, 2). The nucleus of mature neutrophil was highly segmented (3 - 6 lobes) forming ring shape. These lobes were attached to each other by both thin and thick threads of chromatin (Fig. 3 and 4). Band neutrophils were not observed. This was similar to the findings of (10 and 13), and differs from (4) who recorded that band neutrophil was higher in the blood of young than in adult monkeys. This was attributed to the fact that maturation of band neutrophils occurs within central blood circulation and not within peripheral blood. With difficulty, sex chromatin (barr body) can be identified in female neutrophil. It was round dense mass of heterochromatin lies under or near to the nuclear envelope (Fig. 1). This result is similar to that found in (13).

Eosinophils were easily identifiable and similar to those of human. They were smaller than neutrophils (Fig. 5) and (Table, 3). Basophils were the most less frequently observed leukocytes (Table, 2). These results reported that the nucleus of basophil was obscured and covered by numerous basophilic granules within pale blue cytoplasm (Fig. 5 and 6). This result was different from most animal species except cow (13). According to the size of some leukocytes, vervet monkeys had three sizes of lymphocytes; small, medium and large and two types of monocytes (Fig. 5-9).The larger the leukocytes, the more active the cells were. Similar result was reported by (12) who stated that ruminant lymphocytes were thrown into three sizes. As vervets act as a carrier for many viral diseases (20), this explains why lymphocyte: neutrophil ratio was high. Our hypothesis about the presence of numerous sizes of some leukocytes may be attributed to the functional stimulation that occurs within the circulation and not to the maturation and developmental events as the latter occurs in the bone marrow. Present conclusion was similar to the finding of (21) who reported that when lymphocytes were stimulated by any disease, their size will correspondingly enlarge. Besides, the hemopoietic developmental series showed that there was only one developmental stage between lymphoblast and lymphocyte (22). Although lymphocytes and monocytes are easily distinguished from each other by both size and morphology, it is not uncommon to find cells which are intermediate between the

two. The current results referred that there was overlapping and misleading between small monocytes and medium and large lymphocytes (Fig. 6, 8 and 9). This was in agreement with (21 and 23). It seems that the size of circulating monocytes increases due either to chemotactic stimulation or to the process of monocyte phagocytosis. It can be concluded that the different sizes of circulating leukocytes appear to be a transient phase in the functional development of tissue leukocytes (24 and 25).The present study demonstrated that the blood platelets were small irregular bodies, lacking nuclei and lies among blood cells especially erythrocytes. This was in variance partly with (25) who described blood platelets as occur among both, erythrocytes and leukocytes. Blood platelets had different shapes and sizes. They range between 1.5 to 4.15 µm in width (Fig. 10) and (Table, 3). Since red blood cells were highly differentiated, anucleated, devoid of organelles, survive a long period in the circulation and lacking any defense mechanism (21), so that it was susceptible to be infected by many pathogens. (22 and 25) referred to the phagocytic adhesive capability of blood platelets.

Table, 1: The results of RBCs count cell x10^6/µl, related red blood corpuscle values, and blood platelets count cell x10^3/µl in four groups of vervet monkeys, (M±S.E.)

Examined Blood Parameters	Animals Groups			
	Young		Adult	
	Group 1 Males ♂	Group 2Females ♀	Group 3Males ♂	Group 4Females ♀
RBCs x 10^6/µl	6.46±0.14 A	5.77±0.05 B	6.51±0.09 A	5.81±0.09 A
Hb gm/dl	15.43±0.33 A	13.65±0.43 B	15.93±0.11 A	13.83±0.64 A
PCV%	43.7±0.93 A	38.88±0.43 B	45.06±0.36 A	39.5±0.65 C
MCV fl	67.70±0.23 A	67.34±0.16 B	68.46±0.48 A	67.96±0.13 AB
MCH pg	23.90±0.11 A	23.65±0.09 B	24.46±0.17 A	23.79±0.07 B
MCHC gm/dl	35.30±0.26	35.12±0.21	35.34±0.11	35.00±0.15
Blood Platelets x 10^3/µl	2.67±0.08 C	2.79±0.09 A	2.27±0.02 C	2.42±0.02 B

Table, 2: The results of WBCs count, Neutrophil %, Eosinophil %, Basophil %, Lymphocyte %, and Monocyte % in four groups of vervet monkeys.

Examined Blood Parameters	Animals Groups			
	Young		Adult	
	Group 1 Males ♂	Group 2Females ♀	Group 3 Males ♂	Group 4 Females ♀
WBCc x 10^3/µl	7.7±0.11 A	6.98±0.14 B	7.3±0.13 A	6.79±0.12 B
Neutrophil %	26.25±1.55 B	36.5±2.40 A	19.75±5.74 C	41.25±2.46 A
Eosinophil %	3.25±0.25 A	2.75±0.48 A	3.75±0.25 A	3.75±0.25 B
Basophil %	0.5±0.33	0.5±0.33	0.5±0.33	0.5±0.33
Lymphocyte%	59.5±1.44 A	50±1.78 B	61.25±1.49 A	46.5±2.33 B
Monocyte %	11±1.29 B	9.75±0.85 B	14.75±1.25 A	9.5±0.65 B

L.S.D. values: RBCs count=0.3; Blood Platelets count=0.06; Hb=1.1; PCV=1.7; MCV=0.7; MCH=0.3; MCHC=0.

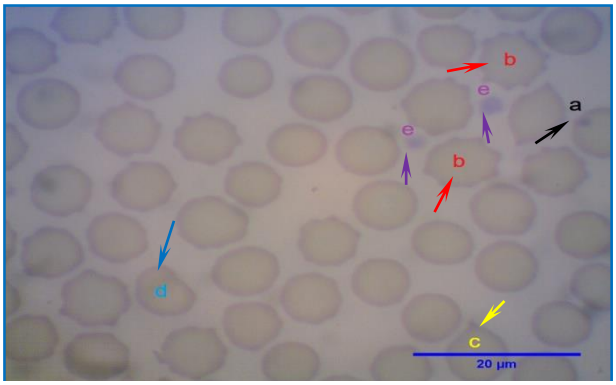
Table, 3: The results of cell diameter for RBC, Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes, and Blood Platelet in µm in four groups of vervet monkeys.

Cell Type		Male ♂	Female ♀
RBC		7.8±0.12	7.8±0.12
Neutrophil		13.19±0.44	13.25±0.44
Eosinophil		12.56±0.36	12.44±0.36
Basophil		13.39±0.32	13.26±0.32
Lymphocyte	Small	8.95±0.14	8.88±0.14
	Medium	10.36±0.16	10.28±0.0.15
	Large	13.01±0.32	13.15±0.32
Monocyte	Small	10.50±0.22	10.25±0.21
	Large	16.00±0.19	15.87±0.18
Blood Platelet		3.02±0.22	3.23±0.23

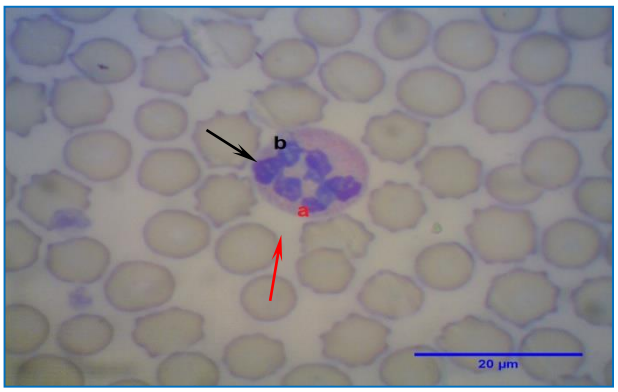
Figures represent mean ± standard error, There are no significant differences between male and female (P>0.05).
Mean cell diameter of 500 cells in smear, N=20.

The current study revealed firstly that there was a reverse relationship between the number of blood platelets and erythrocytes in different ages and sexes. This may be attributed to the conversion role of blood platelets to erythrocytes, supporting, and repair functions. This was confirmed by the results of (26) who reported that under special conditions of blood loss, blood platelets had the ability to transform into hemoglobin-rich respiratory functional erythrocytes, besides, blood platelets function in blood vessels repair. The current work declared that any cellular unit in the body was composed of two types of cells i.e., principle functional and supporting sustentacular cells. Thus, this study regarded the blood platelets to serve as supporting cells for blood cells. This conclusion was consistent by the aggregation of blood platelets that occurs mainly among erythrocytes and not leukocytes (Fig. 3 and 10). Avian thrombocytes may play an important role in innate immunity since these cells have phagocytic capability and can move foreign materials from blood (22, 27 and 28).

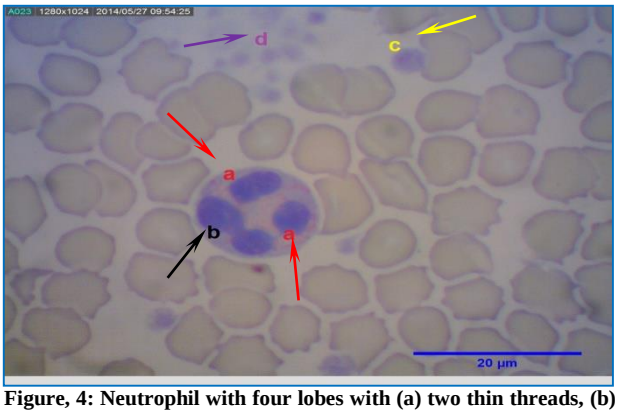
Figure, 1: (a) Female mature neutrophil with sex chromatin, (b) Central Pallor of RBC giemsa stain, (100 x × 1.3 megapixels).



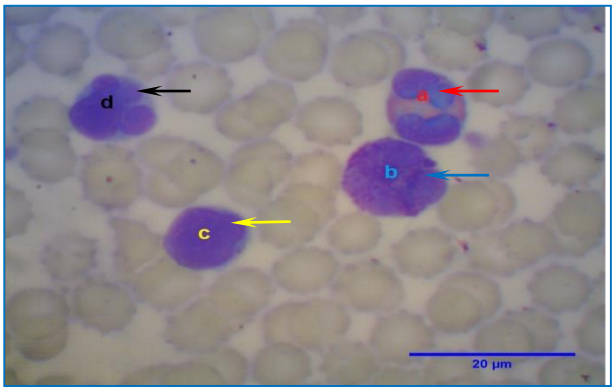
Figure, 2: (a) Howel-Jolly body. (b) Echinocyte shape of RBC. (c) Tear drop shape RBC. (d) Helmet shape RBC. (e) Blood Platelets (100x × 1.3 megapixels).



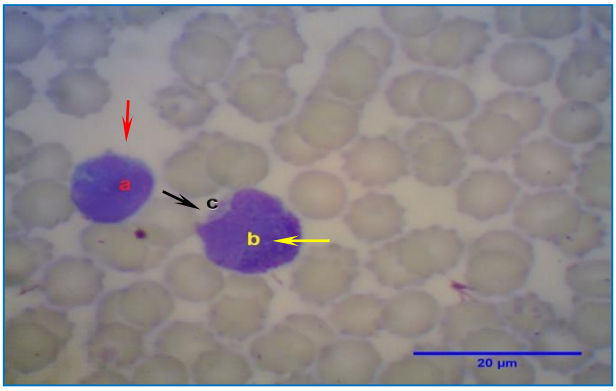
Figure, 3: Neutrophil with six lobes of segmented nucleus (100x × 1.3 megapixels).



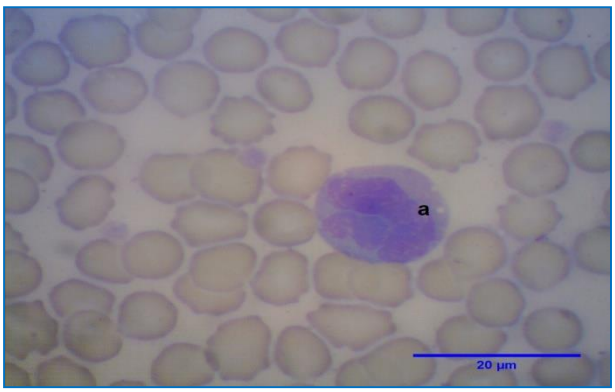
Figure, 4: Neutrophil with four lobes with (a) two thin threads, (b) thick thread, (c) large-sized blood platelet, (d) Aggregation of blood platelets (100x × 1.3 megapixels).



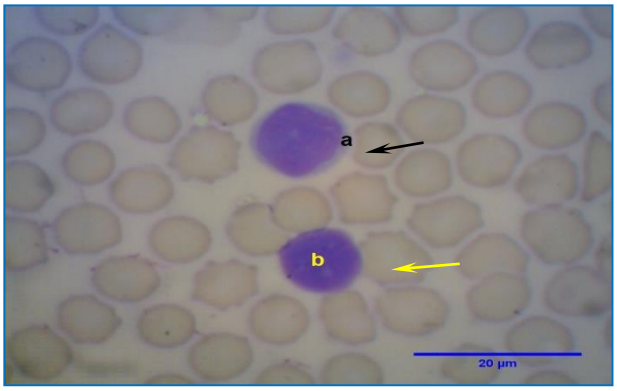
Figure, 5: (a) Eosinophil. (b) Basophil. (c) medium-sized lymphocyte. (d) dividing lymphocyte (100x × 1.3 megapixels).



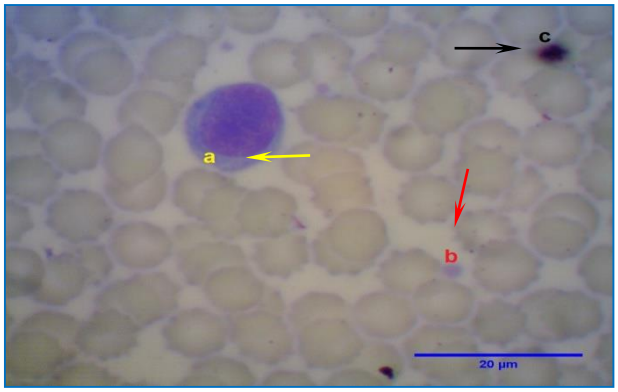
Figure, 6: (a) Small functional monocyte, (b) Basophil, (c) Pale cytoplasm (100x × 1.3 megapixels).



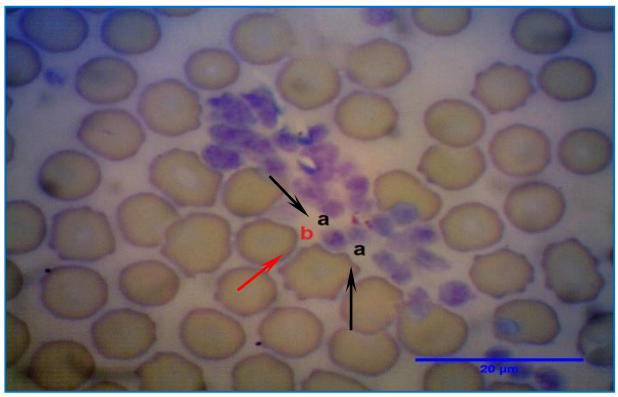
Figure, 7: (a) Large functional Monocyte (100x × 1.3 megapixels).



Figure, 8: (a) Peripheral band cytoplasm of medium-sized lymphocyte, (b) Small-Sized Lymphocyte (100x × 1.3 megapixels).



Figure, 9: (a) Large-sized lymphocyte, (b) Blood Platelet, (c) Artifact (100x × 1.3 megapixels).



Figure, 10: Aggregation of Blood Platelets (a) Lightly stained peripheral hyalomere, (b) darkly stained central granulomere (100x × 1.3 megapixels).

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دراسة نسجية مقارنة للصورة الدموية بين القردة اليافعة والبالغة، الذكور والإناث في جنس (*Chlorocebus pygerythrus*)

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الخلاصة

استعملت في الدراسة عشرين من قردة الفيرفيت لبيان تأثير الأعمار والأجناس المختلفة على الصورة النسجية والدموية. أظهرت الدراسة النسجية أن الكريات الحمر تكون من النوع كبير الحجم كما في الإنسان، والتي احتوت على جسيمات هول-جولي. لم تسجل الدراسة وجود تكوين الرصيص، تفاوت حجم الكريات، التكتل و التراص. بينت الدراسة وجود تأثير للعمر والجنس على الصورة الدموية. أظهرت الذكور البالغة سيادة معنوية في المعايير الدموية. كما في المجترات سجلت الخلايا اللمفية العدد الأكبر بين خلايا الدم البيض. سجلت إناث القردة سيادة في الخلايا العدلة بينما الذكور سيادة في الخلايا اللمفية. تم تشخيص ثلاثة أحجام للخلايا اللمفية وحجمين للخلايا وحيدة النواة. كانت معدلات الخلايا وحيدة النواة الأعلى في الذكور. تميزت الخلية العدلة بكبر حجمها وامتلاكها نواة عالية التفصص حلقية الشكل. لم تسجل الدراسة وجود الخلايا العدلة الشريطية في الدم المحيطي. كانت نسبة الخلايا الحمضة عالية. تعتبر الخلايا البيض الدورانية كطور انتقالي في التطور الوظيفي للخلايا البيض النسجية. أظهرت الأقراص الدموية تعددا في الأحجام، والأشكال والألفة للصبغات القاعدية وطبقا لتلك الألفة الصبغية شخصت الدراسة نوعين من الأقراص: فاتحة غير فعالة و داكنة فعالة. أشارت الدراسة لوجود علاقة عكسية بين أعداد الكريات الحمر و ألأقراص الدموية بين الأجناس و الأعمار المختلفة. ترى الدراسة أن الأقراص الدموية تعمل كتراكيب ساندة لخلايا الدم وخاصة الكريات الحمر. تستنتج الدراسة أن آلية الدفاع المناعية الخلطية في القردة متطورة جدا.

الكلمات المفتاحيه: الصورة الدموية، قردة ذكور، قردة إناث، نسجي.