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Study of Camels RBCs and Elemental Analysis Using Scanning Electron Microscopy (SEM) and Elemental Dispersive Spectroscopy (EDS) in Southern Iraq

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Abstract

The current study was designed to assess some critical mineral elements concentration in the serum of camels using advanced methods like Energy Dispersive Spectroscopy analysis (SEM-EDS) using scanning electron microscope of minute camel's blood. The present study was conducted on forty-eight healthy adult males and females of camel (Camelus dromedarius) aged three to five years in three southern governorates of Iraq: Basra, Thi-Qar and Muthana (6 males and 10 females per governorate). Blood samples were applied to field emission scanning electron microscopy (FE-SEM) to recognize the essential cellular morphological properties found in camel's RBCs that assist camels to adaptation on harsh condition. While, serum was subsequently assayed for elemental analysis by energy dispersive spectroscopy (EDS). The SEM images of RBCs showed a smooth surface with a circumference invagination referred to tropical waist with difference in length and width of RBCs between male and female. While, EDS percentage atomic concentrations study of camel's serum revealed different amount of essential, heavy and rare trace elements in both sexes from the Basra and Thiqar governorate and different levels in essential elements in the serum of both sexes of Thiqar and Muthana governorates.

Keyword: Camels, RBCs, SEM-EDS, Southern Iraq

Introduction

The famous Arabian camels (*Camelus dromdarius*) in Bedouin of Iraq are the Khawar breed which is characterized by a medium sized body, small head, long thin legs, thin short tail, variable hair colors and had ability to stand harsh conditions (1). The Khawar breed is mostly found in Iraq. However, some can be found in Syria, Jordan and Saudi Arabia. The other, Jodi breed is characterized by a high body mass and their herders are mostly distributed in the arid and semi – breed arid areas of southern part of Iraq to Saudi-Arabian border. The Omani breed is also present in Iraq, but in fewer numbers (2).

Arabian camels distributed in Arid and semi-arid areas due to certain anatomical and physiological adaptations. Every system in the camel body has certain modification which make it better adapted to desert life than other animals (3).

Camels are unique among mammals in having small elliptical and non-nucleated red blood cells (RBCs), these cells can swell to twice their underlying volume following rehydration which oppose osmotic variety without cracking (4). Among the special features of the camels which make them superior for traveling long distances and carrying heavy load, their capacity to store both energy and water to tolerate dehydration extremely well, tolerate scanty food, and have the ability to lower their resting metabolism. This may be due to heat balance by remarkable diurnal fluctuation of their body temperature during the hot condition, and lower resting oxygen consumption and energy cost. Camels can tolerate a water loss amount to 30% of its body weight by maintaining blood volume in spit body water loss (5).

Hematology and biochemical enzymes activities are an important clinical veterinarian tools for health management and diagnosis of disturbance in the animals, also provide the opportunity for the clinician to assesst the physiological, nutritional and pathological status of an organ (6). A study conducted in the Camel Research center of King Faisal University in the city of Al-Ahsa, were twenty health camels of each of Majaheem, Maghateer and Awarik breeds were utilized. Study resolved moreover the hematological parameters serum calcium, copper, potassium, sodium and zinc levels. The outcomes covered were no noteworthy in breed and sex impact. Seasonal variation in hemato-biochemical parameters (minerals assayed: Ca, Na, K, Mg, P) in mature female camels were recorded in previous study (7).

Information on the concentration of critical minerals in the serum is crucial pre-requisite for improvement of the mineral deficiencies or toxicities of animal diets for the improved performance. Although camel is an important domestic animal species in countries like Iraq that possesses a large population, little has been done on the estimation of mineral status using modern analysis techniques like scanning electron microscope. Advanced methods like Energy Dispersive Spectroscopy analysis (SEM-EDS) using scanning electron microscope of minute camel's blood can be carried out, therefore, the present study was designed to assess some critical mineral elements concentration in the serum of camels using this method.

Materials and Methods

Peripheral blood samples were collected from the jugular vein and quickly stored in cooled box for transport to the laboratory. Subsequent study was done using scanning

electron microscope for male and female blood camels to compare between both sexes in the same location and also compare the results for the two sexes with their counterparts in other southern governorates.

Electron Microscope Study

The scanning electron microscopy (SEM) was done in the Faculty of Pharmacy, University of Basrah (FE-SEM Unit, Carl Zeiss – Germany), that produce data about the sample including outside morphology (surface), substance arrangement, crystalline structure and introduction of materials making up the sample.

The SEM is also capable of performing chemical microanalysis of selected point locations on the sample. It is especially useful in determining chemical compositions using Energy Dispersive X-Ray Spectroscopy or (EDS). The EDS method recognizes x-rays discharged from the sample and collected by an electron pillar to portray the basic synthesis of the dissected volume (13). The goal was to utilize this modern delicate technique to examine the components in µm zone from the camel's moment blood test including 1 µl (Figure 1). The wet blood sample should be chemically fixed, dehydrated, dried and finally mounted on the SEM stage (22).

Dehydration is accomplished by replacing sample free water with the organic solvent like ethanol, methanol or acetone and finally drying using air or chemical method to mount the sample on adhesive stub of the SEM chamber.

Atypical result of specimen is printed on word program showed the distribution of the elements. A spectrum acquisition showed the characteristic x-rays of the K, L, M series along with normal concentration (norm. C. %), Atomic Concentration (Atom. C. %), and Sigma Weight percentage (Sigma Wt. %) (Figure 2). Statistical analysis:

In EDS study, since sigma weight represents the back scatter electron detector's polarization signal, the important factor in our analysis is the atomic concentration percentage which will be counted and statistically analyzed using t-test analysis (Comparing between two averages). All data were expressed as mean ±SD.

Result

Scanning Electron Microscopy (SEM) of RBCs:

A simple method for preparing erythrocytes for scanning electron microscopy is by sequential fixation with glutaraldehyde and dehydration in a graded series of alcohols. The method allows visualization of membrane defects not seen under the light microscope and therefore is suitable for routine processing of erythrocytes for diagnosis. (19) The RBCs were flat thin wafer like ellipsoidal in shape lacking the nuclei. While, nucleated ones were more round in shape. Scanning electron micrographs showed a smooth surface with a circumference invagination or depression around the short oval axis near the margin forming a waist line around the oval erythrocyte which lacks central paleness. Further calculations were done on RBC SEM images to compare size parameters of both sexes for Basrah governorate which yielded an extremely significant increase ($P \leq 0.001$) of female RBCs longitudinal axis than males, with no significance difference in width ($P \geq 0.05$).

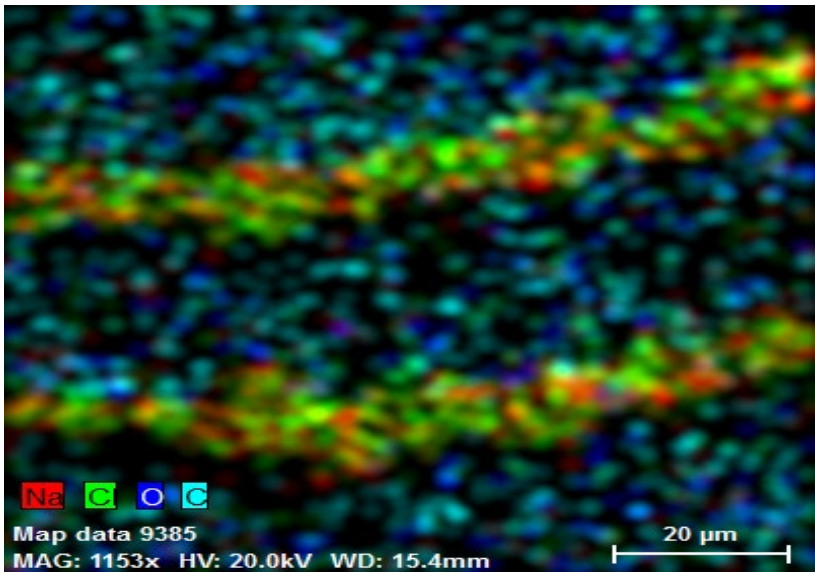

Figure (1): Mapping data using SEM-EDS technique

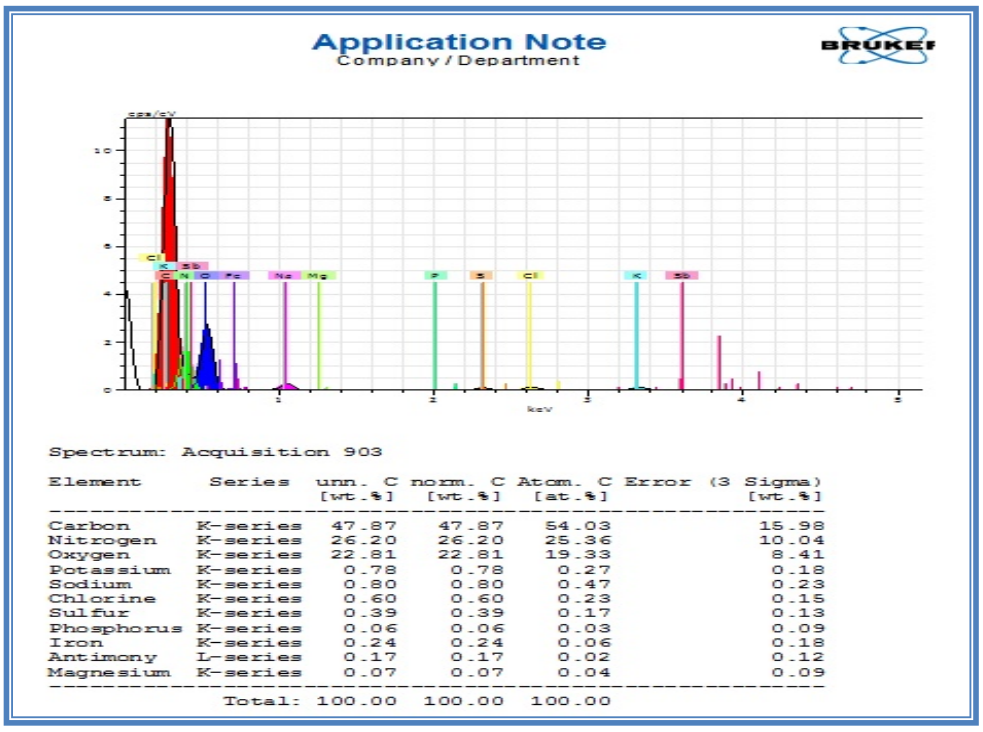

Figure (2) FE-SEM EDS Analysis Result Data Sheet

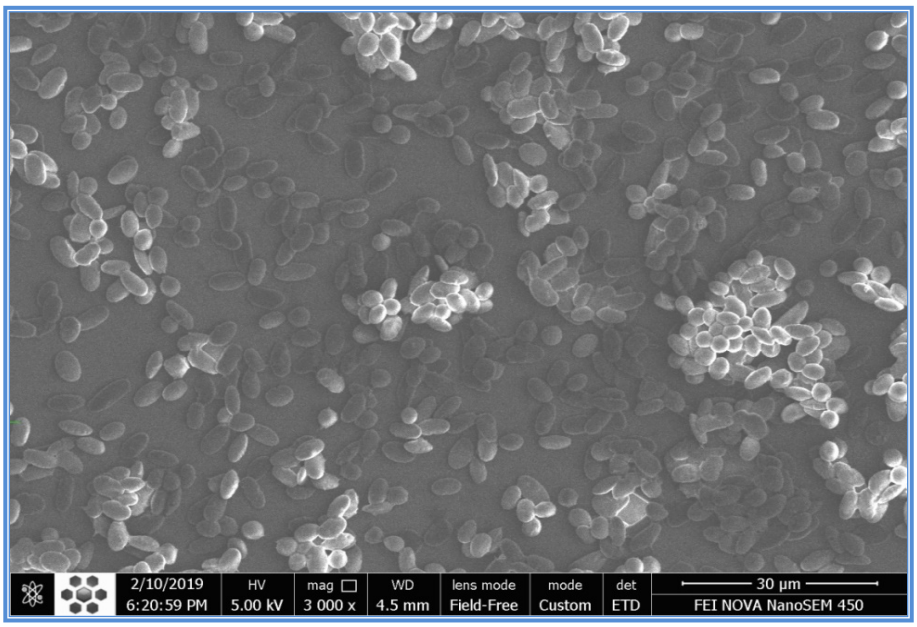

Figure (3): SEM image of male camel RBCs. (3000X)

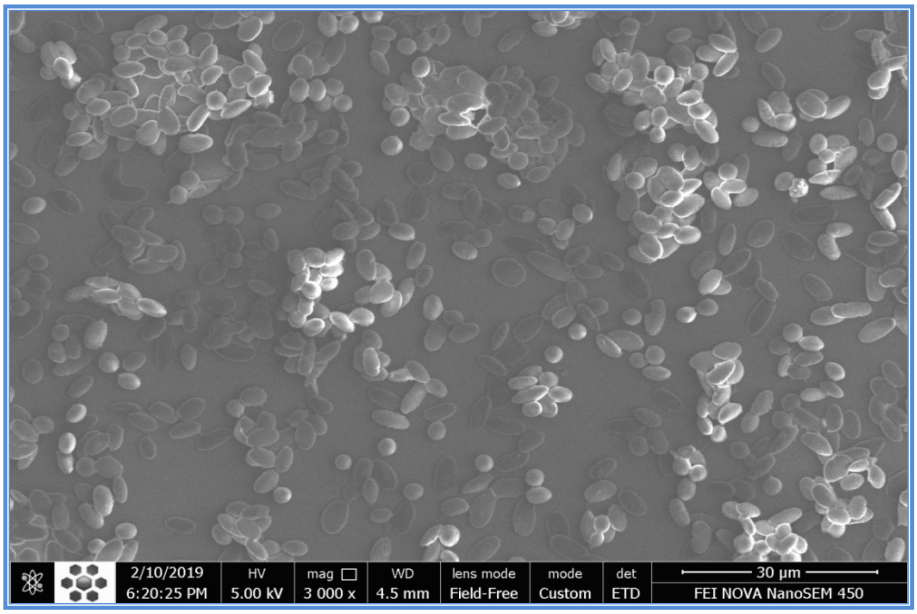

Figure (4): SEM image of female camel RBCs. (3000X)

Male camel RBCs were averaged 7.44 ± 0.81 µm in long with a mean width of 2.7± 0.09 µm. whereas female camel RBCs were averaged 8.64 ± 0.36 µm in long with a mean width of 3.5 ± 0.75 µm. Figure 5 showed SEM image of male camel RBCs and Figure 6 showed SEM image of female camel RBCs. First for Basra governorate, the data were as follows, Table (1) presented the data means ±SD and they were not significant for the carbon, oxygen and nitrogen constituents.

Second, the essential and heavy elements like: Potassium, Chlorine, Sodium, Sulfur, Phosphorus, Magnesium, Iron, Silicone, Selenium, Ytterbium, Lead, Bromine, Samarium, Aluminum and Calcium atomic concentrations (%) ±SD were presented in table 2 and 3. Figure 7 represented each element with its significance value. Atomic concentrations (A.C.) of Basrah male camels were higher than those for female camels for the trace elements potassium, chlorine, sodium and sulfur without significance difference (P≥ 0.05).

On the other hand, the A.C. of phosphorus and iron of male camels were respectively highly significant (P≤ 0.01) and significantly (P≤ 0.05) greater than those for female camels.

Among the essential elements inspected, magnesium A.C. was highly significant (P≤ 0.01) lower in male camels than in females. While, calcium A.C. has been lowered without significance. Antimony and aluminum A.C. showed no significant change or decrease respectively between both camel sexes, nevertheless trace element like silicone A.C. decreased highly significantly (P≤ 0.01) in males than in female camels.

Heavy elements like selenium and lead revealed the A.C. highly significant decreased (P≤ 0.01) in male camels than in female ones. While, the A.C. of ytterbium and samarium revealed decrease significantly (P≤ 0.05) and highly significantly (P≤ 0.01) in males than in female camels, respectively Finally, bromine A.C. showed decrease in male camels than in females without significance (P≥ 0.05). Secondly, for Dhi-qar governorate the data as follows,

Figure 8 presented the data means ±SD for the atomic concentration percentage means for elements in blood Samples from Dhi-qar camels' group. Thirdly, for Muthana

governorate figure 9 presented the data means ±SD for the atomic concentration percentage means for elements in blood Samples constituents from Muthana camels' group.

For comparisons between sex in the same governorateand among the governorates for the same sex, the below table (4) represents Means ±SD of percentage atomic concentrations of five elements EDS values for both sexes in three governorates. percentage of elements C, O, in Dhi-qar and Muthana governorate appeared lower significantly (P≤0.05) in camel than female camel. While, in Basra governorate female camels, A.C. of C increased and A.C. of O decreased but without significance.

The results revealed that A.C. of Na decreased significantly (P≤0.05) in female camels of governorates Dhi-qar and Muthana and insignificantly (p≥0.05) in Basra, which in turn it had extremely decreased (P≤0.01) both camel and female camels Na A.C.The comparison of S A.C. values for both sexes revealed no significant difference between camel and she camels in Basra and Dhi-qar governorates, except Muthana, where it increased extremely significantly (P≤0.01) in female camels.

The A.C. of Cl increased extremely significantly (P≤0.01) in all of camels than female camels of Dhi-qar and Muthana governorates, it insignificantly increased (p≥0.05) in Basra camels, with significant difference between all three governorates. In comparisons between governorates, Basra only had the highest incidence of essential elements like Potassium, Phosphorus, Iron, calcium and Magnesium along with a group of heavy metals like Antimony, Selenium, Lead bromine and Samarium which are markers of polluted grazing areas.

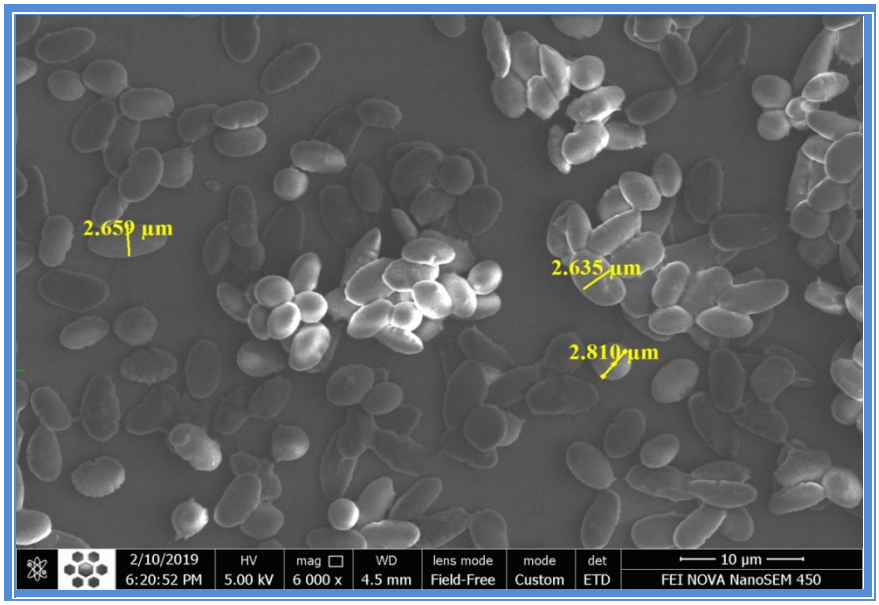

Figure (5): SEM image of male camel RBCs. (6000X)

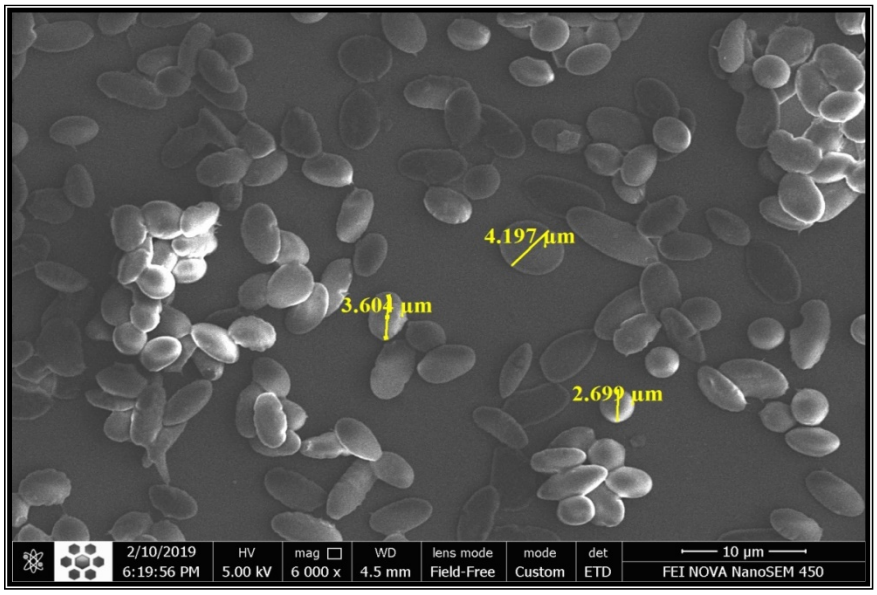

Figure (6): SEM image of female camel RBCs. (6000X)

Table (1): Atomic Concentration Percentage (%) means for elements: C, N, and O in Blood Samples of Basra camels' group

Elements Atomic Concentration% N=20	Carbon	Nitrogen	Oxygen
Camels Mean ±SD	55.603 ±0.8465	24.19 ±0.5841	19.355 ±0.3505
She-Camels Mean ± SD	60.928 ±12.075	17.277 ±10.526	15.053 ±7.2111
P-value	0.33	0.1688	0.204
Significance	-	-	-

(-): Not Significant P>0.05 , (*) : Significant P≤0.05, (**): Highly Significant P≤0.01

Table (2): Atomic Concentration Percentage means for elements K, Cl, Na, S, P, Fe In Blood Samples of Basra camels group.

Trace Element	Potassium	Chlorine	Sodium	Sulfur	Phosphorus	Iron
Camels Mean (%)	0.188	0.148	0.326	0.097	0.008	0.035
±SD	±0.055	±0.048	±0.046	±0.055	±0.012	±0.020
She-Camels	0.1333	0.0933	0.1767	0.0817	0.0017	0.0317
Mean (%) ±SD	±0.094	±0.064	±0.143	±0.043	±0.004	±0.043
P-value	0.076	0.058	0.125	0.046	0.009	0.030
Significance	-	-	-	-	**	*

(-): Not Significant P>0.05 , (*) : Significant P<0.05, (**): Highly Significant P<0.01

Table (3): Atomic Concentration Percentage means for elements Mg, Sb, Se, Yb, Pb, Br, Sm, Al, Ca in Blood Samples of Basra camels group

Trace Element	Potassium	Chlorine	Sodium	Sulfur	Phosphorus	Iron
Camels	0.188	0.148	0.326	0.097	0.008	0.035
Mean (%)	±0.055	±0.048	±0.046	±0.055	±0.012	±0.020
±SD						
She-Camels	0.1333	0.0933	0.1767	0.0817	0.0017	0.0317
Mean (%)	±0.094	±0.064	±0.143	±0.043	±0.004	±0.043
±SD						
P-value	0.076	0.058	0.125	0.046	0.009	0.030
Significance	-	-	-	-	**	*

(-): Not Significant P>0.05, (*): Significant P≤0.05, (**): Highly Significant P≤0.01

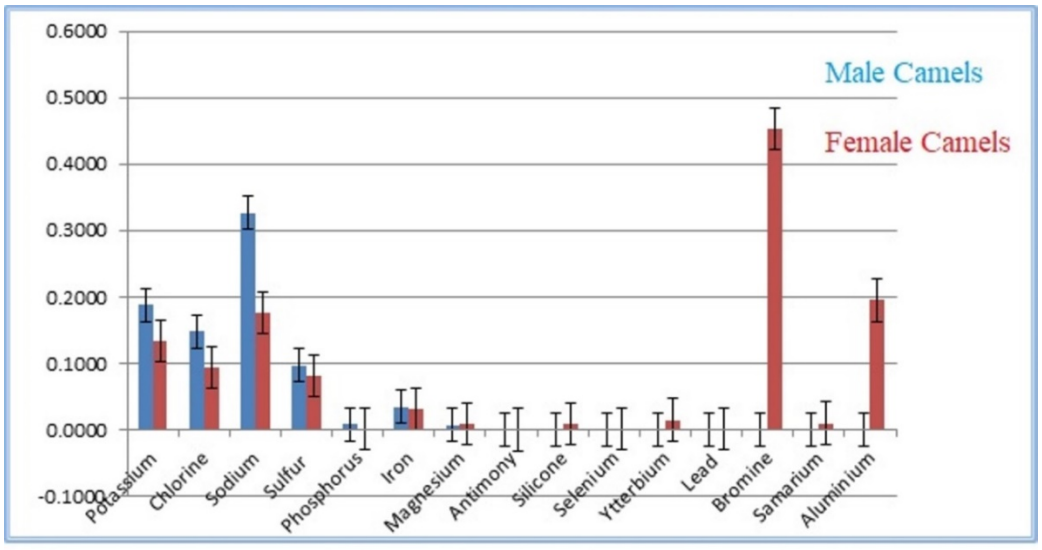

Figure (7): Atomic Concentration Percentage (%) means for elements In Blood Samples of Basra camels' group

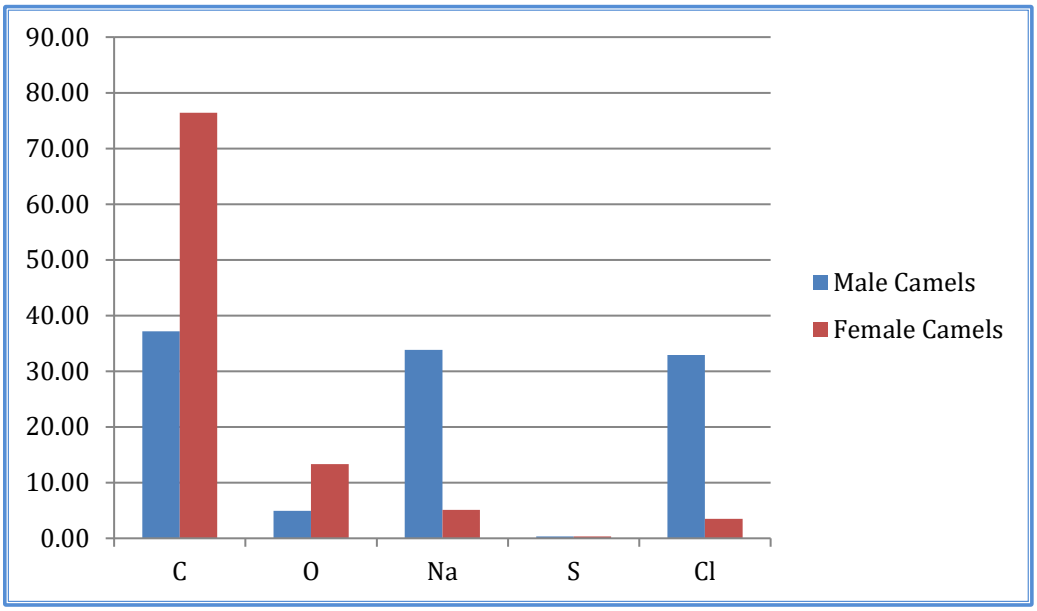

Figure (8): Atomic Concentration Percentage means for elements In Blood Samples from Dhi-qar camels' group

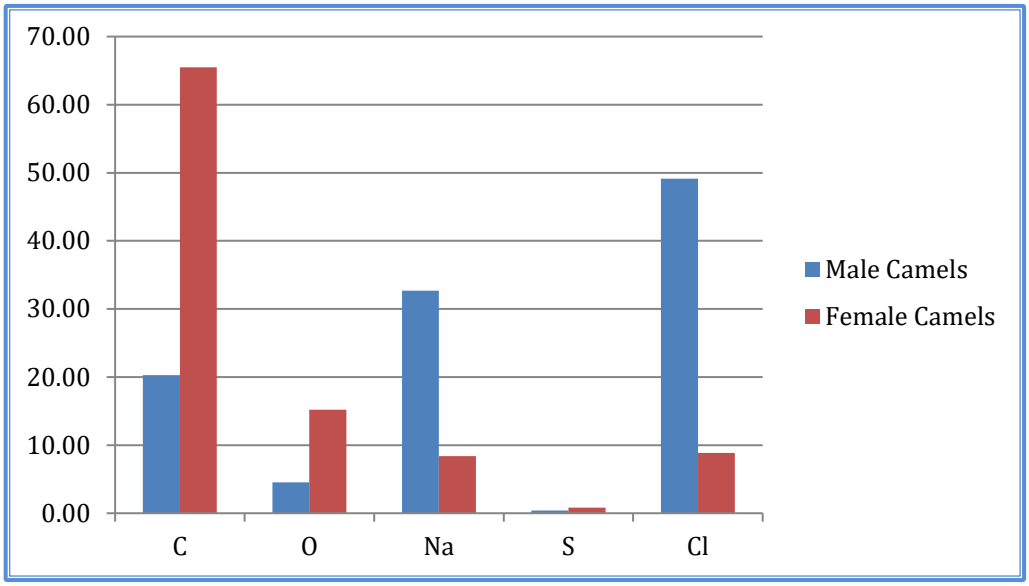

Figure (9): Atomic Concentration Percentage means for elements In Blood Samples of Muthana camels group

Table (4): Means ±SD of percentage atomic concentrations of five elements EDS values for both sexes in three governorates in south Iraq.

Elements	Basra		Dhi-qar		Muthana	
	Male	Female	Male	Female	Male	Female
C	55.60 ±0.84 a	60.92 ±12.07 b	37.15 ±4.58 *ab	76.42 ±2.51 a	20.30 ±3.37 *b	65.47 ±2.80 b
O	19.355 ±0.350 a	15.053 ±7.211 b	4.94 ±0.74 *b	13.33 ±1.56 a	4.55 ±0.96 *b	15.21 ±1.76 b
Na	0.326 ±0.046 a	0.176 ±0.143 a	33.86 ±8.99 *b	5.11 ±1.13 ab	32.66 ±4.22 *b	8.37 ±1.74 b
S	0.097 ±0.055 a	0.081 ±0.043 a	0.34 ±0.04 b	0.36 ±0.06 ab	0.40 ±0.12 *b	0.83 ±0.15 b
Cl	0.148 ±0.048 a	0.093 ±0.064 ab	32.94 ±7.80 *ab	3.52 ±0.77 a	49.14 ±5.41 *b	8.87 ±1.80 b

(*) Refer to significant value ($p<0.05$) between sex in the same location. Small letter refer to significant value ($p<0.05$) among the locations for the same sex

Discussion

Morphology of camel erythrocytes as viewed with the scanning electron microscope can reveal distinctive properties that can assist the cells to endure severe osmotic dehydration. Cohen and his associates (20) found that camels retain primitive or early developing Marginal Bands (MBs) of tubulin, arising from

centrioles. These seem to constrain the erythrocyte's ellipsoid form. The uncurving nature of the tubulin bands, elasticity of a kind, works against the contraction of the cell membrane, typically underlain by spectrum network cytoskeleton, uniform in its contraction properties.

The depth of the constricted but bowed ellipsoid diminishes as a consequence, and the nucleus bulges the biscuit somewhat centrally. It is interesting that the tubulin affects a plane, developing a bilateral symmetry, which works to flatten the cell (13). In camelids the abortion of the nucleus dramatically lowers the cell volume, without allowing the constrained and wafer-like ellipsoid to assume any biconcave, radial shape. This cell, however, has perhaps a minimal volume for an erythrocyte to have. Its osmotic toughness and rich oxygen capacity serve well in the hypoxia of camelids or during dehydration

Our study produced SEM images which were unique in that no coating was required during RBCs preparation; since we are using field emission electron microscopy and therefore 5 K.V. accelerating voltage was fair enough to produce clear and vivid images (Figure 4 to 7) unlike the previous study (21) where blood cells were coated with platinum and gold using sputter with an accelerating voltage of 20 K.V. to get same resolution images of non-nucleated, elliptical erythrocytes and tropical waist, with about 7.0±0.5µm in longitudinal diameter which are close to our findings about RBCs calculations (7.44 ± 0.81µm)

Obviously camel erythrocytes are highly resistant to osmotic hemolysis, being able to expand to 240% of their original volume without rupturing in hypotonic solutions and this may be due somewhat to the shape of camel elliptical erythrocytes, which are oval rather than circular discs seen with other arid mammalian erythrocytes. Previous study suggested that the major proteins of camel erythrocyte membranes are similar to those of cattle and humans, with the major difference being in the major membrane protein "spectrin", which appears to be very tightly bound to the camel erythrocyte membrane at the marginal plane (22). These findings went parallel with our study where the SEM images of RBCs showed an invagination or depression around the short oval axis of the RBC. The researcher also stated that camel erythrocytes undergo a change in shape, from flat ellipsoids to spheres, suggesting an important shape-maintaining role for spectrin in the erythrocytes of camels.

This SEM study illustrated large RBCs with round shape as in Figure 7 from female blood specimen of hydrated camel, a fact which was many years ago proven by previous study (23). Scientist proved that when camels are subjected to dehydration the cell size diminishes and the RBC life span increases but the shape does not change and 4 h after a rapid rehydration the cells increased in size and became rounder, 40% of the cells having a long axis equal to the short axis. These changes were confirmed in hypertonic or hypotonic saline solutions which may add to these properties the

moderately high diffusional water permeability of camelid RBC membrane.

An interesting fact declared years ago by Betticher and Geiser (24), were they suggested that a linear correlation with negative slope between the size of RBC and the maximal urinary solute concentration exists in vertebrates; i.e., animals producing the most concentrated urine have the smallest RBCs. Our study using SEM-EDS technique is quite new modality in precise elemental analysis in very small specimen size (nanoscale), along with taking three-dimensional images of the specimens at the same time, so few researches used this technique and our comparisons with the author's results are descriptive in general form due to changed units of measurements. Camel body contains approximately 5% weight minerals which is subject to change as maturity degree and physical fitness and is essential for proper growth and reproduction. In our study the SEM-EDS method revealed the minute quantities of essential minerals which are basic cellular constituents of the physiology of body cells and fluids as mentioned earlier by Underwood and Suttle (16).

Sharkawy et al. (25) revealed same results of our study in that, significant increase in heavy metals like lead and cadmium elemental concentration in male camel's blood specimens, while iron concentration was significantly high in both male camels' blood and kidneys. Temesgen et .al. (26) reported that the mean concentration of P in the wet and in the dry season was very low compared to values reported in other studies. Rakesh et al. (27) results went in line with previous studies were high rate of excretion and impaired absorption of the electrolytes during digestive orders might have contributed for the decrease in phosphorus content in both camel sexes, in contrast to our current study findings where phosphorus was highly significantly greater in males than female camels.

Basra governorate had suffered plenty of wars and dramatic climate changing, along with massive oil industries and power plant establishments, so industrialization offered additional hazards to the environment surrounding man and animals. Our Study went parallel with Antoniou et al. (17) findings where Dromedaries are grazing animals with a potency of indicating environmental pollution because of the water, food and air they receive lodged by contaminants. As this study showed incidence of heavy metal pollution like Selenium, Lead, Ytterbium and Samarium in serum samples of male and female camels of Basra governorate, the distant territories north as preceding to Dhi-qar and Muthana had less industrialization evolution thus decreasing and almost diminishing levels of pollution with contaminants like: Lead, Aluminum, Silicone and Antimony traces were revealed with no incidence of heavy metals.

In conclusion, advanced techniques like scanning electron microscopy clarified the precise RBC geometry that made adaption on harsh condition. While, energy dispersive spectroscopy analysis (EDS) of camel blood had proven a fast judgment idea of the camel

animal health status and physiologic assessment along with biomarkers of environmental pollution.

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دراسة خلايا الدم الحمراء للجمال والتحليل العنصري باستخدام المجهر الإلكتروني الماسح وتقنية مطيافية تشتت الطاقة

الخلاصـــة

هدفت الدراسة لتقييم شكل خلايا الدم الحمراء ولمعرفة التحليل العنصري لدم ذكور واناث الابل في ثلاث محافظات لجنوب العراق (البصرة وذي قار والمثنى). استخدم ثمانية واربعون من الذكور والاناث البالغة للابل *Camelus drmedarius* (6 ذكور و 10 اناث لكل محافظة) بعمر من ثلاث الى خمس سنوات . جمعت نماذج الدم للابل من الوريد الوداجي مباشرة خلال الفترة من شهر شباط الى شهر نيسان 2018 حيث نقلت النماذج الى المختبر لاجراء التحليلات الدمية. أظهرت صور المجهر الإلكتروني SEM التغيرات المورفولوجية التي لم يتم الكشف عنها بواسطة المجهر الضوئي لكرات الدم الحمراء سطح أملس مع إنبعاج محيطي يشير إلى الخصر الاستوائي مع اختلاف في الطول والعرض لخلايا الدم الحمراء بين الذكور والإناث ، في حين أن دراسة EDS لنسبة التركيز الذري وجدت كميات مختلفة من العناصر الأساسية والثقيلة في مصل الهجن من كلا الجنسين من من محافظة البصرة ومستويات مختلفة في العناصر الأساسية في مصل كلا الجنسين في محافظتي ذي قار والمثنى.