Biochemical and Hematological Characterization of Iron Deficiency Anemia in Camels of Anseba and Gash-Barka Regions of Eritrea

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Abstract:

Iron deficiency anemia was studied and characterized in 24 camels of Anseba and Gash Barka regions. Twelve camels were investigated from each region; six young camels below one year old and the other six camels were adult camels above one year old. The aim of this study was to identify anemia caused from iron deficiency in camels using different haematological and biochemical parameters. The investigation of iron deficiency anemia was based on erythrocytic count and morphology, hematocrit estimation and iron determination (both bound and unbound). Blood samples for this study were collected in heparinized test tubes. Erythrocytic count and morphology were studied with the help of Hemocytometer and Giemsa stained blood films, respectively. Iron level was determined using UV-spectrophotometer. Thirteen (54%) out of 24 camels, were found to be anemic. Of these, 7 camels were from Anseba region, and the rest were from Gash Barka. Of the anemic camels, eight were identified as young and the remaining 5 were adult. The study shows that iron deficiency anemia was prevalent in both regions, being slightly higher and severer in Anseba, particularly in poor conditioned young male camels. Although the real cause of iron deficiency anemia was not extensively determined, the results shows alarming situation of poor nutritional and health monitoring of camels in both regions of Eritrea where study was conducted and with high population of camels and hence demanding coherent, long-term strategy for progressive control.

Key words: Anemia; Clinical chemistry; Camels; Iron deficiency; Hematology; Eritrea

1. Introduction:

Camel is an even-toed ungulate under the genus *Camelus*. Three species of camels are found in the world; single humped camel (*Camelus dromedarius*), double humped camel (*C. bactrianus*) and wild Bactrian camel (*C. ferus*). Single humped camel is commonly known as Dromedary or Arabian camel. They are the smallest of the three species. The dromedaries are well-known for transportation, racing, milk, meat and fibre. They can adapt easily in desert region due to their ability to remain without water for extremely long periods and having fluctuating body temperature (Fayed, 2001). They are well-equipped to survive, produce and work in harsh environment (Wu et al., 2014). Ninety four percent of the world's camel population is of dromedary type. Dromedaries are usually found in the Horn of Africa, Middle East and South Asia. The largest concentration of camel in the world is found in the Horn region of Africa alone (Bernstein, 2009). Despite the socioeconomic importance of camels in Eritrea, the animals are suffering from malnutrition related diseases. Accordingly, this paper studies naturally occurring iron deficiency anaemia in some camels of Anseba and Gash Barka region.

Anaemia, is usually defined as a decrease in the total amount of red blood cells (RBCs) or haemoglobin or both in the blood (Williams and Wilkins, 2006). It can also be defined as a lowered ability of the blood to carry oxygen (Rodak, 2007). In slowly developing anemia, the symptoms are often vague and may include feeling tired, weakness, shortness of breath or a poor ability to exercise. Additionally, clinical anemia characterized by

increased pulse rate, sweating, sunken eyes (due to dehydration) and emaciation. A significant form of anemia must be seen before an animal becomes noticeably pale. Additional signs may occur depending on the underlying causes (Janz *et al.,* 2013).Based on causes, there are three main types of anemia, these are; anemia due to blood loss, due to increased red blood cells breakdown (hemolysis) and due to decreased red blood cell production. Causes of decreased red blood cells production include iron deficiency, lack of vitamin- B12 and a number of neoplasms of the bone marrow. Our main focus of study is anemia due to iron deficiency. Iron is a fundamental element for human and animals. Iron exists in the living body as ferritin and hemosiderin which are considered to be proteins containing iron .Iron participates in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. Iron deficiency is one of the leading risk factors for disability and death worldwide, affecting a large number of animal species. Anemia and iron deficiency anemia must always be taken into consideration for they have a clear impact on the patient's quality of life and they can be the consequence of severe diseases (Lippard and Berg, 1994).

Iron deficiency is associated with impairment of cell mediated immunity and the bactericidal activity of neutrophils, thereby increasing the susceptibility to infections. Iron deficiency might play an important role in defence mechanism and thus, the term "nutritional immunity" was coined to highlight the importance of iron deficiency to prevent bacterial growth (Chandra, 1973). The blood profile of camels in Eritrea is poorly documented and little is known about the normal ranges of the biochemistry and haematology blood references compared to the extensive studies conducted in other countries such as Tunisia (Ben Romdhane et al., 2003), Algeria (Aichouni et al., 2010; Ahmed et al., 2013; Aichouni et al., 2013), Morocco (Bengoumi et al., 1997a), Egypt (Abd-El-Salam et al., 2008; Saleh et al., 2009; El-Harairy et al., 2010; Osman et al., 2015), Sudan (Omer et al., 2006; Amin et al., 2007; Dowelmadina et al., 2012; Babeker et al., 2013), Nigeria (Mohammed et al., 2007), Kenya (Kuria et al., 2006) among others. The aims of this research study was to establish a base line reference values for blood biochemical and haematological parameters in normal, healthy, one humped camels commonly found in Eritrea and to highlight the effect of age on these parameters. The mean values of selected biochemical and haematological blood parameters in twenty four (24)

camels (twelve adults and twelve young camels) from lowland regions of Eritrea i.e. Anseba and Gash-Barka regions are presented and compared with the reference values for healthy camels from (Elitok B. and Cirak A.C., 2018).

2. Materials and Methods:

2.1. Study area :

The study was conducted in Sub Zoba Hamelmalo and Mensura, in Zoba Anseba and Zoba Gash Barka, respectively in the state of Eritrea. These areas were selected for sample collection as they are known typically for poor or scarcity of pasture. In addition to that the majority of Eritrean camels are found in these areas (western lowlands).

2.2. Sample collection:

A total of twenty-four blood samples were collected from jugular vein of camels of the above listed areas in heparinised vaccutainer test tubes. Twelve of these were from Anseba region, while the rest (twelve samples) were from Gash Barka region. Each group consisted six young and six adult camels. Five to seven ml of blood sample were aseptically collected from each animal.

2.3. Materials and Reagents:

All the chemicals and reagents were of analytical grades and were obtained from HAC and Azel pharma plc laboratories.

2.4. Morphology of red blood cells:

The morphology was studied using Giemsa stain. Blood samples were collected from jugular vein in to test tubes with heparin. Blood films were prepared fixing the slides in methanol and stained with Giemsa for 15 minutes. The slides were then thoroughly washed, dried and examined under oil emersion lens.

2.5. Total count of red blood cells:

RBC pipette (in the hemocytometer) was filled with well mixed blood up to 0.5 mark. The excess blood on the sides was wiped out. Normal saline (diluting solution) was sucked up to 101 marks. The contents were mixed by rotating the pipette between fingers. First 2-3 drops of fluid were discarded from diluting pipe. Next Neubauer's chamber was charged by touching the tip of pipette at the junction of chamber and cover glass. Finally after waiting for 2 minutes, all the cells in four corner and middle small squares were counted using high power objective.

2.6. Haematocrit estimation:

Whole blood was collected in test tube with Heparin and placed in hematocrit capillary tubes (75*1mm). This blood was used to fill micro-hematocrit tubes, one end was plugged with sealant (wax). Then the hematocrit tubes were centrifuged at 3000rpm for 5 minutes with plugged-end facing outward. The PCV was measured by hematocrit reader, where the bottom of the RBC layer should be at the 0-line and the top of the plasma should be on the top-line. Finally, the percentage was estimated as the line level with the top of the RBC layer.

2.7. Spectrophotometric determination of serum and haemoglobin iron:

Standard iron solution containing 1.25g of iron per 500 Ml for serum samples (unbound iron) and 0.25g for Hb sample (bound iron) of iron per 500ml was prepared by weighing equivalent mass of pure ferrous ammonium sulphate $[Fe(NH_4)_2(SO_4)_2.6H_2O]$ (FW=392.14); i.e. 8.75g for serum determination and 1.75g for Hb determination. The ferrous ammonium sulphate was then dissolved in about 200 mL of distilled deionized water (DDH_2O) containing 5.0 mL of 6 M H_2SO_4 in a 500-mL volumetric flask. This solution was then diluted to the mark with DDH_2O and mixed thoroughly by inverting the stoppered flask several times. From this stock solution, 50 mL was transferred to a labelled plastic reagent bottle. Using calibrated autopipet, 5.0mls of this standard iron solution was then transferred into a 100mls volumetric flask, followed by addition of 10 mL of freshly prepared acetate buffer (5.0 M $HC_2H_3O_2$, 0.5 M $NaC_2H_3O_2$) was added followed by 10mls of freshly prepared 10% w/v hydroxylamine hydrochloride ($NH_2OHCHCl$). The solution was then left to stand for about 10 minutes for the reduction of any Fe^{3+} to be completed. Thereafter 10 mL of 0.1%w/v of 1, 10-phenanthroline solution was added and the solution diluted to the mark with DDH2O and mixed thoroughly until reddish-orange colour developed.

A blank solution was also prepared in exactly the same manner as above but with omission of the standard iron solution. Absorbance was then measured at 510nm using UV-Spectrophotomer.

Four additional standard solutions containing 1ml, 2mls, 3mls and 4mls of the standard iron solution was also prepared in similar manner as described above and their absorbance also determined at 510nm with UV-Spectrophotomer.

Blood samples was also prepared in the same way and their absorbance also measured at 510nm using UV-Spectrophotometer.

A plot of absorbance versus concentration using the data obtained from standard solutions was then constructed, and using equation from linear graph of $y = mx + c$, the gradient m and c constant were also calculated.

This constants were then used to determine the concentration of iron in blood samples against the absorbance obtained from the Spectrophotomer readings.

3. Results and Discussion:

3.1 Results:

A total of twenty four camels were included in this study, twelve were from Anseba and the rest were from Gash Barka region. Each group (zoba) consists six young ($\leq$ 4 years) and six adult (>4 years) camels. The main parameters evaluated were; RBC morphology and count, PCV estimation and iron determination; in RBC (bound iron) and serum (unbound iron). Based on the above listed biochemical and hematological parameters; Seven of Anseba camels were anaemic, meaning that they recorded below the average standard reference values. Five of them were young and the rest were adult camels. Six of Gash Barka camels were anaemic (three young and three adult). Moreover, the detailed information about the general health status of the animals is explained in their respective group tables and figures below.

Table 1: Summary of biochemical and hematological parameters of young camels in Hamelmalo sub zone, Anseba region of Eritrea

Parameters			Camels				Overall Mean	Ref Range (c)
	YA1	**YA2**	**YA3**	**YA4**	**YA5**	**YA6**		

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Sex	M	M	M	M	F	M	N/A	N/A
RBC Morphology	Normocytic, Normochromic	Microcytic, Hypochromic	Microcytic, Hypochromic	Normocytic, Hypochromic	Normocytic, Hypochromic	Normocytic, Normochromic	N/A	N/A
PCV (%)	26.65 ± 1.23^c	23.54 ± 2.22^c	30.34 ± 2.42^a	27.56 ± 0.55^a	28.05 ± 0.54^a	27.66 ± 0.31^a	27.3	**27-45**
RBC count ($\times10^6\mu L^{-1}$)	13.75 ± 0.71^a	10.15 ± 1.34^c	12.77 ± 0.43^b	12.63 ± 0.21^b	10.61 ± 1.67^c	13.51 ± 1.21^a	12.23	**13-18**
MCV (gL^{-1})	10.85 ± 3.56^a	15.67 ± 2.12^a	22.67 ± 1.22^b	18.45 ± 0.18^a	16.43 ± 2.43^a	25.55 ± 2.11^c	18.27	**21-28**
MCHC (gL^{-1})	33.75 ± 1.35^c	30.54 ± 2.24^c	24.35 ± 3.21^c	38.55 ± 2.12^b	37.87 ± 1.56^b	45.67 ± 3.11^a	35.12	**43-48**
USIC ($mgdL^{-1}$)	10.85 ± 3.11^a	15.67 ± 2.11^a	25.14 ± 2.15^b	18.51 ± 2.02^c	24.48 ± 1.54^c	32.61 ± 2.04^a	21.21	**50-200**
BHbI ($mgdL^{-1}$)	10.85 ± 2.12^c	15.67 ± 0.32^b	14.97 ± 1.11^b	19.27 ± 2.11^a	13.83 ± 1.14^b	17.32 ± 0.32^a	15.31	**20-50**
TIBC ($mgdL^{-1}$)	21.7 ± 3.71^c	31.34 ± 3.01^c	40.11 ± 3.61^b	37.78 ± 1.02^b	38.31 ± 1.12^b	49.93 ± 3.32^a	36.52	**70-250**
Comment on Health Status	**Poor**	**Poor**	**Poor**	**Poor**	**Poor**	**Fair**	N/A	N/A

Means on the row with different superscripts letters are significantly different (P≤0.05) for a specified hematological parameter.
YA = Young Anseba camels. N/A = Not applicable
(c) = Citation: Elitok B. and Cirak A.C., 2018

Table 2: Summary of biochemical and hematological parameters of young camels in Gash-Barka region of Eritrea; Sub zone Mensura.

Parameters	YG1	YG2	Camels YG3	YG4	YG5	YG6	Mean	Ref Range [(c)]
Sex	M	M	M	F	F	F	N/A	N/A
Cell Morphology	Normocytic, Hypochromic	Normocytic, Normochromic	Microcytic, Hypochromic	Normocytic, Hypochromic	Normocytic, Normochromic	Normocytic, Normochromic	N/A	N/A
PCV (%)	22.34 ± 3.23^c	29.87 ± 1.55^a	24.45 ± 3.01^c	28.43 ± 1.67^c	32.76 ± 2.11^b	31.24 ± 2.25^b	28.18	**27-45**
RBC count (μL^{-1})	12.11 ± 1.12^c	12.87 ± 1.23^c	14.25 ± 1.12^a	13.54 ± 0.24^b	14.55 ± 1.25^a	14.52 ± 1.27^a	13.64	**13-18**
MCV (gL^{-1})	11.44 ± 3.35^c	22.32 ± 2.65^b	18.87 ± 2.75^b	24.56 ± 2.11^a	24.54 ± 3.01^a	20.23 ± 0.22^b	20.32	**21-28**

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MCHC (gL⁻¹)	28.65±3.16ᶜ	46.87±2.11ᵃ	40.44±1.15ᵇ	40.55±1.01ᵇ	44.34±2.55ᵃ	33.12±3.01ᶜ	38.99	**43-48**
USIC (mgdL⁻¹)	23.55±3.14ᶜ	54.56±2.12ᵃ	44.12±2.77ᵇ	46.98±0.02ᵃ	42.33±2.85ᵇ	25.51±3.11ᶜ	39.50	**50-200**
BHbI (mgdL⁻¹)	13.63±3.33ᶜ	34.87±2.45ᵃ	15.23±3.11ᶜ	23.56±1.03ᵃ	25.34±1.56ᵃ	18.56±2.15ᵇ	21.86	**20-50**
TIBC (mgdL⁻¹)	37.18±4.14ᶜ	89.43±2.44ᵃ	59.35±2.15ᵇ	70.54±2.03ᵃ	67.67±0.55ᵇ	44.07±3.44ᶜ	61.37	**70-250**
Comment on Health Status	**Poor**	**Fair**	**Poor**	**Fair**	**Fair**	**Poor**	N/A	N/A

Means on the row with different superscripts letters are significantly different (P≤0.05) for a specified hematological parameter.

YG = Young Gash-Barka camels. N/A = Not applicable

(c) = Citation: Elitok B. and Cirak A.C., 2018

Table 3: Summary of biochemical and hematological parameters of adult camels in Anseba region of Eritrea; Sub zone Hamelmalo.

Parameters	AdA1	AdA2	Camels AdA3	AdA4	AdA5	AdA6	Mean	Ref Range [(c)]
Sex	F	M	F	M	F	F	N/A	N/A
RBCs Morphology	Normocytic, Normochromic	Normocytic, Normochromic	Normocytic, Normochromic	Normocytic, Normochromic	Microcytic, Hypochromic	Normocytic, Normochromic	N/A	N/A
PCV (%)	35.34±1.32ᵇ	31.56±2.33ᶜ	42.78±3.54ᵇ	33.23±0.15ᵃ	33.86±0.05ᵃ	28.23±2.25ᶜ	33.932	**27-45**
RBC count (µL⁻¹)	12.41±2.44ᶜ	14.23±1.04ᵇ	16.65±3.01ᵃ	14.47±0.05ᵇ	11.32±2.55ᶜ	15.06±2.55ᵃ	14.346	**13-18**
MCV (gL⁻¹)	23.45±2.11ᵇ	19.54±2.04ᶜ	27.76±3.12ᵃ	23.44±1.04ᵇ	18.45±2.01ᶜ	17.53±2.77ᶜ	21.344	**21-28**
MCHC (gL⁻¹)	40.45±2.43ᶜ	41.44±2.12ᶜ	45.55±1.87ᵃ	42.34±1.04ᵇ	40.45±1.87ᶜ	46.41±3.25ᵃ	43.238	**43-48**
USIC (mgdL⁻¹)	52.23±0.55ᵇ	35.33±3.54ᶜ	100.23±3.88ᵃ	44.56±1.55ᵇ	25.45±3.95ᶜ	52.87±0.22ᵇ	51.688	**50-200**
BHbI (mgdL⁻¹)	23.43±2.55ᶜ	21.34±3.14ᶜ	48.65±3.01ᵃ	30.45±0.05ᵇ	16.76±3.85ᶜ	38.44±0.95ᵃ	31.128	**20-50**
TIBC (mgdL⁻¹)	75.66±1.35ᵇ	56.67±2.44ᶜ	148.88±3.55ᵃ	75.01±2.44ᵇ	42.21±3.54ᶜ	91.31±0.43ᵇ	82.816	**70-250**
Comment on	**Fair**	**Poor**	**Excellent**	**Fair**	**Poor**	**Fair**	N/A	N/A

Health Status								

Means on the row with different superscripts letters are significantly different (P≤0.05) for a specified hematological parameter.

YG = Young Gash-Barka camels. N/A = Not applicable

(c) = Citation: Elitok B. and Cirak A.C., 2018

Table 4: Summary of biochemical and hematological parameters of adult camels in Gash-Barka region of Eritrea; Sub zone Mensura.

Parameters	AdG1	AdG2	Camels AdG3	AdG4	AdG5	AdG6	Mean	Ref Range [c]
Sex	M	F	M	F	F	F	N/A	N/A
Cell Morphology	Microcytic, Hypochromic	Normocytic, Normochromic	Normocytic, Normochromic	Normocytic, Normochromic	Normocytic, Normochromic	Normocytic, Normochromic	N/A	N/A
PCV (%)	22.23±3.75^c	23.12±3.63^c	30.84±0.63^b	29.67±0.55^b	30.68±1.41^b	39.04±3.07^a	29.26	**27-45**
RBC count (μL^{-1})	11.05±1.55^c	10.31±2.08^c	10.92±2.13^c	12.11±0.43^b	16.72±3.04^a	16.53±3.01^a	12.94	**13-18**
MCV (gL^{-1})	15.47±2.52^c	12.34±1.55^c	24.43±2.66^b	13.12±2.53^c	27.45±3.83^a	23.44±2.23^b	19.37	**21-28**
MCHC (gL^{-1})	42.23±0.43^b	43.12±1.11^b	45.34±2.41^b	32.23±3.53^c	47.65±3.46^a	44.56±1.43^b	42.52	**43-48**
USIC ($mgdL^{-1}$)	23.54±3.53^c	18.65±4.12^c	44.21±1.43^b	34.55±2.93^c	121.34±4.44^a	75.56±3.53^b	52.97	**50-200**
BHbI (mgdL-1)	15.87±3.41^c	15.54±3.43^c	25.65±2.11^b	20.31±2.85^b	48.77±3.43^a	43.76±2.88^a	28.31	**20-50**
TIBC (mgdL-1)	39.41±3.63^c	34.19±3.83^c	69.86±2.66^b	54.86±2.89^c	170.11±3.63^a	119.32±3.43^a	81.29	**70-250**
Comment on Health Status	**Poor**	**Poor**	**Fair**	**Poor**	**Excellent**	**Good**	N/A	N/A

Means on the row with different superscripts letters are significantly different (P≤0.05) for a specified hematological parameter.

YG = Young Gash-Barka camels. N/A = Not applicable

(c) = Citation: Elitok B. and Cirak A.C., 2018

3.2 Discussion:

The hematological data obtained from young camels in Anseba region shows that most of these camels have general poor health. All the parameters recorded below the average standard reference values apart from camel YA6 that had all parameters within the reference limit even though at lower level and therefore indicating fair health status. The other five young camels were therefore considered to be having poor health status. The hematological data obtained from young camels in Gash-Barka region shows that half of these camels have general poor health while the other half have fair health status. Even though most of the parameters were within

the reference normal values, the values were at the lower scale within the limit. Two male young camels and one female young camels however, recorded general poor health as per the general reference normal scale. Two adult camels from Anseba region had poor health status in reference to analyzed biochemical and hematological parameters. Three others had considerable fair health status while one adult camel was found to have excellent health status with all the determined parameters within the reference range. Only camel AdA6 had most of the determined hematological parameters below the standard reference values. Two female adult camels from Gash-Barka region were found to be generally healthy in reference to the evaluated biochemical and hematological parameters. Camel AdG5 had an excellent health status while camel AdG6 had general good health. However, two other female camels AdG2 and AdG4, and one male camel AdG1 had poor health status in reference to evaluated hematological parameters. Camel AdG3 was considered to be fairly healthy in reference to the analysed parameters.

4. Conclusion and Recommendation:

4.1 Conclusion:

The results of the study indicate that iron deficiency anemia in camels was a common finding in both Anseba and Gash-Barka regions, being slightly higher and severer in Anseba camels. Furthermore, poor body conditioned young camels were more vulnerable to anemia compared to adults. Young male camels were severely affected compared to females.

4.2 Recommendation:

Based on observations and results obtained by our study, the following points are recommended:

(i) We believe that this kind of study is the first of its nature in Eritrea and therefore further studies on the biochemical and haematological parameters on these camels from other parts of Eritrea is required to ascertain the health status of these important animals.

(ii) Studies of seasonal effect on the occurrence of iron deficiency anaemia will be very helpful.

(iii) Good nutritional management practices e.g. administration of iron supplements may be helpful to reduce the burden of this type of anaemia.

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