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Research Article

Serum Iron, Calcium, Phosphorus and Magnesium Concentrations and Their Effects on Hemato-immune Dynamics in Diseased Camels (*Camelus dromedarius*)

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Abstract

The research study was aimed to measure serum iron, calcium, phosphorus and magnesium concentrations and to determine their association with hemato-immune status in diseased camels. A total of 48 adult dromedary camels (*Camelus dromedaries*) were classified into 3 groups. Group 1: Included twenty apparently healthy camels free from external and internal parasites showed no clinical signs of illness act as control; Group 2: Included 12 camels infected by *Trypanosoma evansi*; Group 3: Included 16 camels suffered from Mange. Red blood cells count, hemoglobin and packed cell volume were significantly decreased in diseased group (Group 2 and Group 3) compared to Group 1 ($P < 0.05$), white blood cells count, neutrophils %, monocytes % in group 2 were significantly elevated than group 1 and group 3 ($P < 0.05$). Iron, phosphorus, calcium and magnesium levels were significantly diminished in group 2 compared to group 1 and group 3 (P value < 0.05). Red blood cells were significantly decreased in diseased camels with low phosphorus, magnesium and iron concentrations ($P < 0.05$). Additionally, white blood cells were low in diseased camels with low iron concentrations compared with these camels with normal iron concentrations. While, no association was found between low calcium with blood and immune response in diseased camels ($P < 0.05$). In conclusions, low calcium, iron, magnesium and phosphorus concentrations were associated with altered hemato-immune status and also have clinical relevance with poor outcome in diseased and poor conditioned camels.

Keywords: *Camel; Trypanosomiasis; Mange; Iron; Phosphorus; Magnesium; Calcium*

Introduction

Camel can survive for many days without water and food, and this distinctive camel capacity is perfect for those rough circumstances that are also frequently referred to as the Desert Ship. Although camel is a significant part of a group of animals that produce food, in the form of milk and meat, for human consumption it is still the most disregarded in scientific research [1]. There are around 28 million camels globally, including 24 million in Africa, four million in Asia and only seven thousand in Europe [2].

Approximately 94% of the world's estimated camel population are one-humped or dromedary camels, while 6% are two-humped Bactrian camels which is located in Asia, animal's body temperature, breathing, heart and pulse are significant physiological elements and, in reality, an index of health and disease [3].

Trypanosomiasis is an important disease of camels in Egypt [4], which is mechanically transmitted by biting flies such as *Tabanus, Stomoxys* and *Lyperosia* [5] and characterized clinically by fever, anorexia, widespread edema, anemia, anemic mucus membrane and quickly deteriorating and dying [6]. *Sarcoptes scabiei var cameli,* a camel ectoparasite, a highly pruritiate and contagious skin disease [7], transmitted through close physical contact with contamination or infected livestock [8] and characterized by irregular alopecia, intense pruritus, exuding dermatitis, scab formation, erythematosus skin and the skin becomes dark and thick [9]. Hematology became an instrument for diagnosis and management increasingly important in veterinary medicine worldwide. The animal's blood image given a clinical chance to explore the existence of various metabolites and other components in the animal's body and played a crucial role in evaluating the organism's physiological, nutritional and pathological status [10]. Biochemical blood component analysis can often assist the clinician with the provision of normal reference values to facilitate animal health and sickness evaluations [11]. Inflammatory conditions either due to infection or to tissue injury cause a number of host reactions including fever and cardiovascular system stimulation and changes in the serum levels of certain trace metals during these conditions (e.g. a reduction in serum iron and phosphorus concentrations) [12]. Iron exists mainly in the blood in erythrocytes as hemoglobin as well as in plasma transferrin. It is transported as transferrin, stored as ferritin or hemosiderin, and lost by bleeding and sloughing cells [13]. Iron is a major component of succinate dehydrogenase and a part of heme of hemoglobin (Hb), myoglobin and cytochromes [14]. The preservation of the RBC physiological levels depends on a subtle balance of RBC uptake with RBC generation and iron homeostasis [15]. In cellular metabolism, magnesium is a very important component and a needed cofactor for several enzymes. This proposed that hemolytic anemia was caused by energy metabolism disturbances in the RBC [16].

It has been reported iron, phosphorus and magnesium is essential for hematological and immune response against disease, however, this association need further investigation in diseased camels. Therefore, the aims of our study were to measure serum iron, calcium, and phosphorus and magnesium concentrations and to investigate their relationship with health outcomes in diseased camels.

Materials and Methods

Animal's criteria

A total of 48 adult male and female dromedary camels (*Camelus dromedaries*) were examined in separate farms and separate locations in Menofia and Behera Governorates, Egypt and covered a variety of 2-8 years and 200 - 550 kg body weight (BW) between September 2018 and May 2019. The examined camels were received a mixture of silage, hay, roughage and clover. According to general health conditions, body condition score and physical examination of the camels, they were split into 3 groups. Group 1: Included twenty apparently healthy camels free from external and internal parasites showed no clinical signs of illness; Group 2: Included 12 camels infected by *Trypanosoma evansi*; Group 3: Included 16 camels suffered from Mange showed abnormal lesions on skin surfaces.

Clinical information

The initial general clinical examination was performed by techniques outlined by [17]. Body condition scoring in camels was a visual and tactile evaluation of body fat reserves and was carried out according to [18].

Samples

Blood was collected from each animal via jugular vein puncture and was divided into two aliquotes. The first aliquot was added to a tube containing 5 mg ethylenediaminetetraacetic acid (EDTA) for adopting hematological examination. The samples were kept in an ice box containing crushed ice and were transferred to the laboratory for immediate procedure. The second aliquot was collected in dry clean centrifuge tube and kept in sloping position without agitation till coagulation. The clotted samples were centrifuged at 3000 rpm for 10 minutes for separation only clear non hemolysed sera for biochemical analysis.

One the same day of collection, fecal samples which were collected individually in the covered cups were examined by direct smear for the detection of gastrointestinal parasites and flotation technique using solution of saturated salt [19].

Blood film examination and skin scrapping

Thin, air-dry, fixed blood smears were prepared, stained with Giemsa-stain and tested microscopically on light microscope (40X and oil immersion objectives) for blood parasites demonstration [20]. For the mange mite demonstration, skin scraping blade samples were taken from suspected cases of mange mite infestation by scratching the lesion edge until capillary bleeding had been seen and maintained by 10% formalin [21].

Hematological and biochemical measurements

The hematological examination involving red blood cells count (RBCs), hemoglobin (Hb), packed cell volume (PCV), Mean corpuscle volume (MCV), Mean corpuscle hemoglobin (MCH), Mean corpuscle hemoglobin concentration (MCHC), white blood cells count (WBCs) and differential leukocytic count were conducted by methods described by [22].

Total protein and albumin have to be measured in serum using Bio diagnostic company's commercial kits. Globulin was calculated by removing the value of albumin from total protein value and division of albumin concentration into globulin concentration calculated A/G ratio according to [23]. Commercial kits were used for spectrophotometric determination of urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), iron (Fe), phosphorus (P), calcium (Ca), and Magnesium (Mg) (Bio-Diagnostic, Giza, Egypt).

Statistical analysis

Data from healthy and diseased camels were compared by means of one-way ANOVA by using the statistical package for social science (SPSS) for windows (Version 16.0; SPSS INC., Chicago, Ill) [24]. Serum iron, phosphorus, calcium and magnesium concentrations were further categorized based on their concentrations from healthy camels into low and normal concentrations. Results were expressed as the mean ± standard error (SEM). Cut-off values of calcium and magnesium, iron and phosphorus concentrations in diseased camels were determined based on their values from the apparently healthy ones. Area under curve (AUC) was calculated with Receiver Operating Curve (ROC) by using GraphPad Prism 8. Significance was considered at $P < 0.05$.

Results and Discussion

Group 1: Included twenty apparently healthy camels free from external and internal parasites showed no clinical signs of illness by inspection or physical examination. Group 2: Included 12 camels with various clinical signs such as fever, anorexia, abdominal edema, dullness, superficial lymph node enlargement, emaciation and paleness of mucus membrane with appearance of pin pointed petechiae (Figure 1A). Giemsa- stained blood smears revealed abnormal presence of T. evansi between red blood cells. Group 3: Included 16 camels suffered from appearance of abnormal lesions on skin surfaces especially in areas of neck, thigh and flank region, the skin become dry and hard (Figure 1B), area of alopecia with crust formation, intense pruritis and itching. Examination of skin scrapings in 20 per cent potassium hydroxide revealed the presence of Sarcoptic scabiei var. cameli. Similar findings have been reported by [25]. Clinical picture of camel trypanosomiasis such as dullness, tachycardia, anemic mucus membrane, edema in brisket and eyelids have been attributable to anemia which was caused by *Trypanosoma* sp, anorexia may be attributed to persistent fever in infected camels with trypanosomiasis; furthermore, lymphoid hyperplasty at an early point could explain the enlargement of superficial lymph nodes [26].

Body condition score can be useful in detection of potential problems that might cause a decrease in camel production. Apparently healthy camels in group 1 characterized by good body condition score BCS ≥ 3 as the hump was extremely dominant, while Group 2 and group 3 suffered from poor body condition score BCS ≥ 1, BCS ≥ 2, respectively (Table 1), indistinct hump in camels in group 2 (Figure 1C).

Group	Group 1	Group 2	Group 3
BCS features	The hump sacs contain enough fat to create a hump, presence of swelling beyond the width of the transverse processes. Flank regions are highly apparent, hollow cavity around Ano-genital region, BCS ≥ 3	The hump was indistinct; the hump sac contains little or no fat, severe degrees of emaciation with clearly visible ribs, Hollow appearance of the flank, more visible cavity around Agno-genital region. BCS ≤1	The hump was present and the hump sac contains enough fat to create a distinct shape, visible ribs, visible cavity around Ano-genital region, BCS ≤2

Table 1: *Body condition score (BCS) in apparently healthy camels (Group 1) and diseased camels (Group 2 and Group 3).*

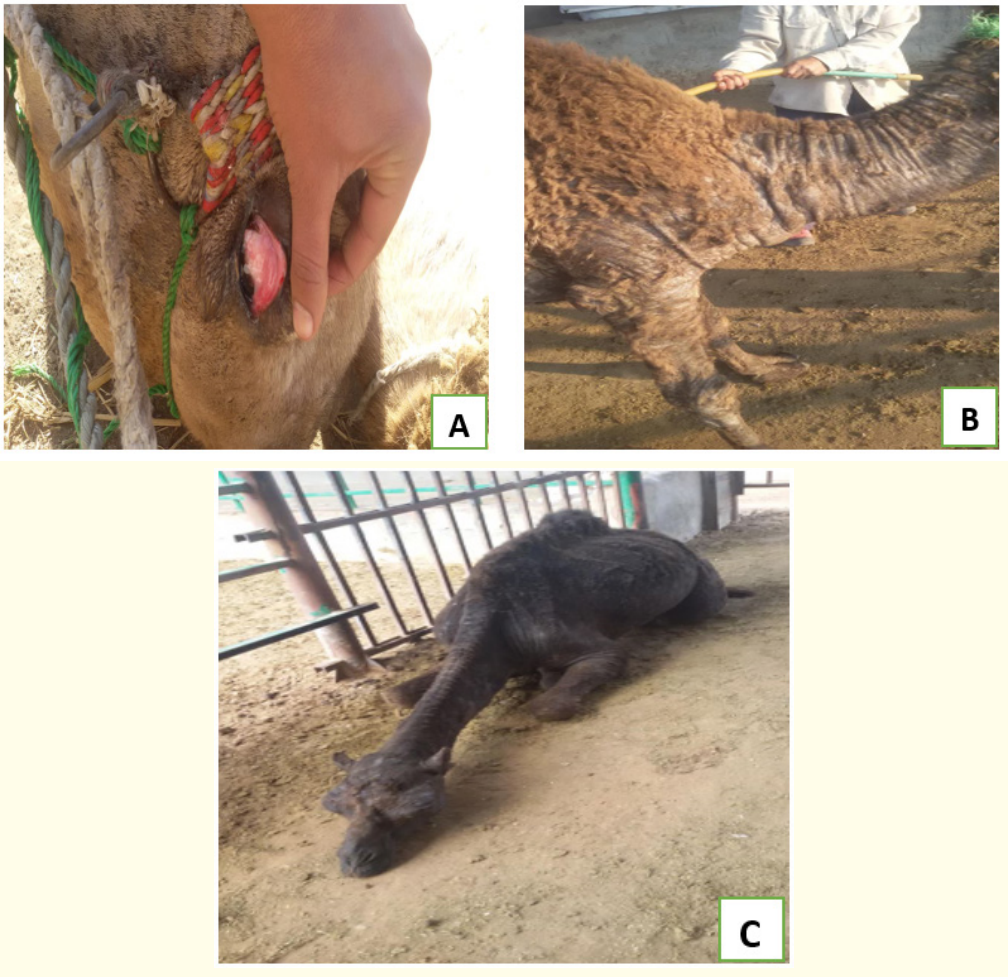

Figure 1: *(A) Diseased camel showed anemic mucus membrane with petechiae. (B) Showed Mange lesions on skin surfaces, skin become dry and hard. (C) Showed poor BCS.*

Although the mean values of the body temperature, respiratory rate and pulse rate were significantly increased in group 2 compared to apparently healthy camels and group 3 (P value < 0.05), the mean values of ruminal motility showed significant decrease in group 2 (P value < 0.05) than apparently healthy ones, and on the other hand the mean values of the body temperature, respiratory rate and pulse rate were not significantly changed for group 3 in comparison to group 1 (P value > 0.05) except for ruminal motility showed significant decrease than group 1 (P value < 0.05) (Table 2). These results were nearly similar to that obtained by [27], the mean values of the body

temperature, respiratory rate and pulse rate were significantly increased in trypanosomiasis which was the same with the parasites presence in peripheral blood or may be contributed to presence of secondary bacterial infection, sluggish of ruminal motility might be attributed to digestive disturbance that leads to ruminal stasis [26].

Variables	Group 1 (n = 20)	Group 2 (n = 12)	Group 3 (n = 16)
Body temperature (°C)	37.52 ± 0.09^{a}	39.64 ± 0.225^{b}	37.36 ± 0.14^{a}
Respiratory rate (cycle/min)	12.55 ± 0.3033^{a}	17.318 ± 0.3239^{b}	12.44 ± 0.4279^{a}
Pulse rare (beat/min)	33.15 ± 0.6252^{a}	43.75 ± 0.7^{b}	31.56 ± 0.6388^{a}
Ruminal motility (movement/2min)	4.25 ± 0.1428^{a}	1.00 ± 0.2673^{b}	3.125 ± 0.1548^{b}

Table 2: *Clinical examination in apparently healthy camels (Group 1) and diseased camels (Group 2 and Group 3) (MV ± SE). n: Number, means within the same row having the different superscripts differ significantly different at (P < 0.05).*

In the current study, the mean values of the red blood cells count, hemoglobin and packed cell volume were significantly decreased in diseased group (Group 2 and Group 3) compared to apparently healthy ones (P < 0.05) and there is no statistical significant variation between group 2 and group 3 (P value > 0.05), nevertheless MCV, MCH and MCHC values showed no statistical significant variations among all groups (P value > 0.05) (Table 3). Moreover, red blood cells were decreased in diseased camels with low phosphorus, magnesium and iron concentrations (Figure 2). Almost comparable findings have been reported by [28] in camels infected with trypanosomiasis, and Comparable results recorded by [29] in camels with sarcoptic mange. Mechanisms for anemia development include: hemolysis, free fatty acids, immunologic mechanisms, and dilution of blood, disorders of clotting, erythrogenic depression and trypanosomal sialidase release [30]. During trypanosomiasis, sialidase is the most significant oxidative enzyme, sialic acid is hydrolyzed by sialidase in the erythrocyte surface membrane [31]. Decline in hemoglobin and red blood cells count values may be caused by blood loss from hemorrhagic lesions of skin attributable to mite burrowing [32].

Variables	Group 1 (n = 20)	Group 2 (n = 12)	Group 3 (n = 16)
RBCs (10^{6}/µl)	7.955 ± 0.1752 a	6.35 ± 0.11^{b}	6.356 ± 0.12^{b}
Hb (g/dl)	12.46 ± 0.2486^{a}	10.23 ± 0.229^{b}	9.80 ± 0.177^{b}
PCV (%)	31.7 ± 0.398^{a}	25.125 ± 0.61^{b}	25.87 ± 0.762^{b}
MCV (fi)	40.16 ± 0.923^{a}	39.61 ± 1.022^{a}	40.85 ± 0.765^{a}
MCH (pg)	15.73 ± 0.336^{a}	15.5044 ± 0.53^{a}	15.503 ± 0.398^{a}
MCHC (%)	39.36 ± 0.8491^{a}	40.76 ± 0.917^{a}	37.99 ± 0.758^{a}
WBCs (10^{3}/µl)	14.95 ± 0.25^{a}	20.25 ± 0.94^{b}	13.15 ± 0.155^{c}
Neutrophil (%)	57.80 ± 0.7276^{a}	66.13 ± 1.394^{b}	58.94 ± 1.066^{a}
Lymphocyte (%)	35.85 ± 0.754^{a}	25.13 ± 1.63^{b}	32.63 ± 1.11^{a}
Monocyte (%)	4.55 ± 0.153^{a}	5.25 ± 0.3134^{b}	4.563 ± 0.128^{a}
Eosinophil (%)	1.65 ± 0.15^{a}	3.5 ± 0.378^{b}	3.813 ± 0.2276^{b}
Basophil (%)	0.15 ± 0.08^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Table 3: *Hemogram and leukocytic counts in apparently healthy camels (Group 1) and diseased camels (Group2 and Group 3) (MV ± SE). n: Number, means within the same row having the different superscripts differ significantly different at (P < 0.05).*

In our research study the mean values of the white blood cells count, neutrophils %, monocytes % in group 2 were significantly elevated than group 1 and group 3 ($P < 0.05$), white blood cells count values in group 3 were significantly diminished than group 1 and group 2 ($P < 0.05$), and also decreased in diseased camel with low iron concentrations (Figure 2D), while the mean values of eosinophil % were significantly increased in group 2 and group 3 in comparison to group 1 ($P < 0.05$), on the other hand the mean values of basophil % were not significantly changed ($P > 0.05$) (Table 4), the almost identical outcomes were documented by [33]. The increase of mononuclear phagocytic system activity in trypanosomiasis results in leukocytosis, neutrophilia could be attributed to secondary bacterial infection in diseased camel [34], specific parasite diseases and allergic reactions may also lead to eosinophilia [35].

Variables	Group 1 (n = 20)	Group 2 (n = 12)	Group 3 (n = 16)
Iron (µg/dl)	116.3 ± 1.554 [a]	85.52 ± 0.864 [b]	94.71 ± 1.676 [c]
Phosphorus (mmol/l)	3.532 ± 0.082 [a]	1.994 ± 0.047 [b]	2.878 ± 0.057 [c]
Calcium (g/dl)	2.629 ± 0.065 [a]	1.379 ± 0.083 [b]	2.247 ± 0.04 [a]
Magnesium (mmol/l)	1.069 ± 0.029 [a]	0.7975 ± 0.028 [b]	1.038 ± 0.03 [a]

Table 4: *Minerals profile in apparently healthy camels (Group 1) and diseased camels (Group2 and Group 3) (MV ± SE). n: Number, means within the same row having the different superscripts differ significantly different at ($P < 0.05$).*

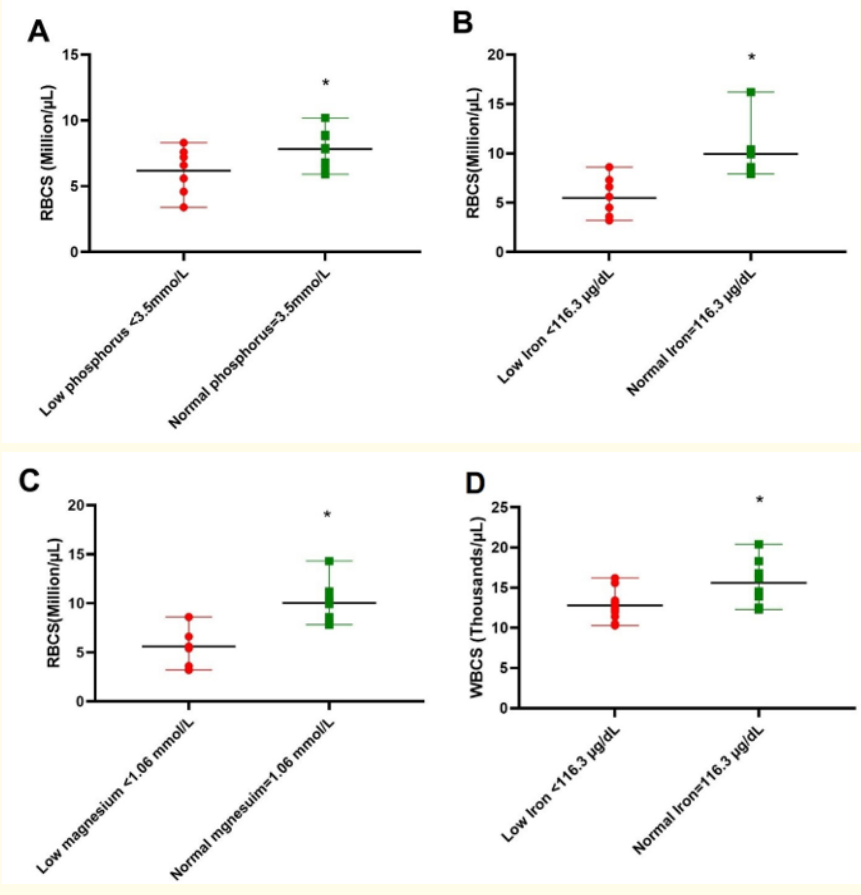

Figure 2: *Red blood cells and white blood cells based on phosphorus, iron and magnesium concentrations in diseased camels. * indicates statistically significant at $P < 0.05$.*

The mean values of the iron and phosphorus were significantly diminished in group 2 compared to group 1 and group 3 (P value < 0.05), and the mean values of these elements (iron and phosphorus) were significantly lower in group 3 in comparison with apparently healthy camels in group 1 (P value < 0.05), Although calcium and magnesium values were significantly lower in group 2 compared to group 1 and group 3 (P value < 0.05), the mean values of these elements (calcium and magnesium) were not significantly changed between apparently healthy camels in group 1 and diseased camels in group 3 (P value > 0.05). Significant decrease in the concentration of iron was discovered to be considered as a defense mechanism in the body prohibited by introducing iron into the cells [34]. Trypanosomiasis caused anemia is a disorder that is linked to a disturbed iron homeostasis including iron recycling by macrophages and iron sequestration by erythrophagocytosis [36]. The reduction of phosphorus level might have been due to hyporexia with consequent lower intake of phosphorus or renal wasting [37]. Hemolysis associated by magnesium deficiency was directly suggested by the intense erythroblastosis [16]. Hypocalcemia in infected camels with surra disease could be due to decreased protein level, insufficient dietary intake, intestinal disturbance and renal failure while decreased phosphorus concentration due to diarrhea and renal wasting [38].

In the present study, although the mean values of the total protein were significantly higher in group to and group 3 compared to group 1 (P value < 0.05), albumin and A/G ratio values in group 2 were significantly changed than group 1 and group 3 (P < 0.05), the mean values of globulin were not significantly changed. On the hand there were statistical significant changes among group 3 and group 1 (P < 0.05) (Table 5). Additionally, low A/G ratios were associated with low concentrations of calcium and iron in diseased camels (P < 0.05; Figure 3) which could indicate their role in reduced immune response in diseased camels. Values of serum urea and creatinine were significantly higher in affected camels with trypanosomiasis than infested camels with sarcoptic mange and apparently healthy ones (P < 0.05), while there were not statistical significant changes between group 1 and group 3 (P > 0.05), activities of liver enzymes including AST and ALT were significantly higher in group 2 than group 1 and group 3 (P < 0.05) and the mean values of the liver enzymes (AST and ALT) were significantly decreased than group 2, and significantly elevated than group 1 (P < 0.05) (Table 5). The nearly same results recorded by [33]. Diminished levels of serum proteins can be caused by extravascular fluid protein in body cavities caused by the diseased edema of the lymph nodes [39]. Significantly elevated values of liver enzymes such as, AST and ALT were also reported in camels infected with trypanosomiasis. These high liver enzymes outcomes could be caused by centrilobular degeneration owing to hypoxia and serious oxidative stress caused by parasitic infection [40].

Variables	Group 1 (n = 20)	Group 2 (n = 12)	Group 3 (n = 16)
Total protein (g/dl)	7.292 ± 0.1359 [a]	6.25 ± 0.129 [b]	6.46 ± 0.738 [b]
Albumin (g/dl)	4.477 ± 0.091 [a]	3.45 ± 0.115 [b]	3.86 ± 0.058 [c]
Globulin (g/dl)	2.815 ± 0.132 [a]	2.79 ± 0.13 [a]	2.71 ± 0.12 [a]
A/G ratio	1.684 ± 0.125 [a]	1.25 ± 0.08 [b]	1.47 ± 0.082 [c]
Urea (mmol/l)	7.107 ± 0.165 [a]	12.34 ± 0.4895 [b]	7.276 ± 0.207 [a]
Creatinine (mg/dl)	0.859 ± 0.027 [a]	1.613 ± 0.048 [b]	0.966 ± 0.382 [a]
AST(U/L)	84.51 ± 0.47 [a]	109 ± 0.97 [b]	96.65 ± 2.3 [c]
ALT(U/L)	14.6 ± 0.49 [a]	25.64 ± 1.3 [b]	20.1 ± 0.577 [c]

Table 5: *Biochemical profile in apparently healthy camels (Group 1) and diseased camels (Group2 and Group 3) (MV ± SE). n: Number, means within the same row having the different superscripts differ significantly different at (P < 0.05).*

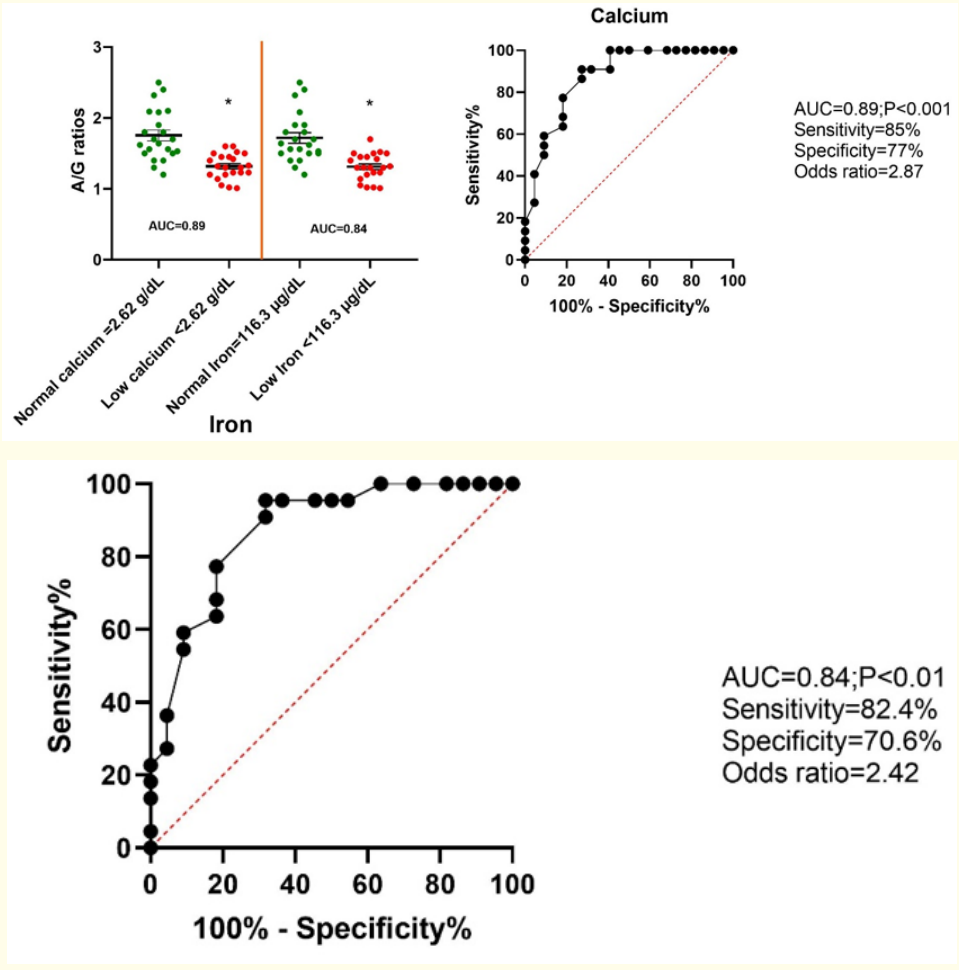

Figure 3: *Receiver operating curve (ROC) of the association of low calcium and iron concentrations with A/G ratios in diseased camels.*

Conclusion

There is a direct relationship between diminished levels of serum iron, and phosphorus and magnesium concentrations and hemato-immune status alterations in diseased camels which will need rapid correction to improve health and immune status of diseased camels during hospitalization.

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Conflict of Interests

No conflict of interests was declared.

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