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Serum vitamin A and E, copper, zinc and selenium concentrations and their relationship with health outcomes in dromedary hospitalized camels (*Camelus dromedarius*)

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Abstract

The goals of this study were to measure serum vitamin A (retinol) and E (α -tocopherol) and trace elements concentrations (copper, zinc and selenium) during diseases condition and to determine their association with hematological parameters and immune status of hospitalized camels. A total of 95 dromedary camels [healthy (n=65); hospitalized camels (n=30)] were included in this study. Vitamin A and E concentrations were significantly lower in hospitalized camels than apparently healthy ones ($P<0.05$). Hospitalized camels had lower concentrations of zinc and selenium compared to healthy camels ($P<0.05$). Vitamin E, copper, zinc and selenium concentrations were positively correlated with phagocytic activity in hospitalized camels ($P<0.05$). The likelihood of deficiency of vitamin A and E, zinc and selenium concentrations were significant in female hospitalized camels than males and in young age hospitalized camels < 6 years old compared to old ones ($P<0.05$). Decreased vitamin A and E and trace elements concentrations were associated with hospitalized camels' phagocytic activity and index. The prevalence of low vitamin A and E, zinc and selenium concentrations were frequent in female hospitalized camels and hospitalized camels of age < 6 years old suggesting severe oxidative stress.

Keywords: Camel, Copper, Selenium, Vitamin, Zinc.

Introduction

Vitamins and trace elements are needed in small amount for vital functions in many animal species including energy production, enzyme activity, resistance against diseases and their deficiencies result in immunological dysfunction and other metabolic disorders (Underwood and Arthur, 1996). Low vitamin A and E, copper, zinc and selenium concentrations lead to many problems eg. decreased reproduction and oxidative stress in farm animals including camels; however, their association with the immune status of camels requires further investigation.

Vitamin A plays essential role in epithelization of many organs and immunomodulation against infection. Additionally, it has been reported that vitamin A is deficient in feeding regimen of the farm animals therefore, its dietary supplementation is very necessary to the ruminant diet (Duester, 2008; Baraka, 2012; Pourjafar *et al.*, 2014).

Vitamin E is also involved in many physiological processes against free radicals and poly unsaturated fatty acids that in turn prevent muscular and cellular injury and oxidative stress (Bennett and Brown, 2003; Baraka, 2012).

Trace elements including copper, zinc and selenium had an important antioxidants function; they also play a role in maintaining normal oxidants/antioxidants and immunomodulation (Hambidge, 2003; Nayyar and Jindal, 2010; Pourjafar *et al.*, 2014).

Of interest, vitamins and trace elements is a contributory factor in the process of phagocytosis that categorized into chemotaxis (migration of phagocytes to inflammatory sites), attachment of particles to the cell surface of phagocytes, ingestion (phagocytosis) and intracellular killing by oxygen-dependent (oxidative burst) and oxygen independent mechanisms (Roitt *et al.*, 1996; Bhaskaram, 2002; Mehrzad *et al.*, 2014). This raises a question whether vitamin A and E and trace elements status will have impact on phagocytic index and phagocytic activity in hospitalized camels. Moreover, serum concentrations of vitamin A, E and micro-elements based on sex and age require further investigation in poor conditioned hospitalized camels during admission time.

The aims of this study were to determine vitamin A, E, copper, zinc and selenium status during diseased conditions of dromedary hospitalized camels and to determine their association with their immune status

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and health outcomes. We hypothesized that vitamin A, E, copper, zinc and selenium concentrations will be frequent finding during time of hospitalization and associated with their health outcomes.

Materials and Methods

Animals' criteria

A total of 95 adult camels (*Camelus dromedarius*), of different age and sex were involved in this study. The camels were divided into two main groups. Group 1: Included 65 apparently healthy camels and their health status were confirmed by physical examination in the farm and their body condition score (BCS=3-5) by inspection and manual palpation (Crabb *et al.*, 1990; Faye *et al.*, 2001). Group 2: Included 30 hospitalized camels admitted to veterinary clinics at different area at Menofia Governorate suffered from many pathological conditions including Gastrointestinal affections (n=8), rough hair coat (6), lacrimation (n=5), coughing with nasal discharge (n=7), and ticks with pale mucus membrane (n=4) and were confirmed by physical examination and their BCS= 1-2 due to pathological conditions during time of hospitalization (Crabb *et al.*, 1990; Faye *et al.*, 2001).

Hospitalized camels (Group 2) were further divided into subgroups based on vitamins and trace elements concentrations. Subgroup 2.1: included 10 dromedary camels with lower blood serum values of copper and selenium (↓Cu, Se). Subgroup 2.2: included 4 camels with lower blood serum values of copper and vitamin A (↓Cu, vitamin A). Subgroup 2.3: Included 7 camels with lower blood serum values of copper, zinc and vitamin E (↓Cu, Zn, vitamin E). Subgroup 2.4: Included 4 camels with lower blood serum values of vitamin E and zinc (↓vitamin E, Zn). Subgroup 2.5: Included 5 camels with lower blood serum values of vitamin A and selenium (↓vitamin A, Se).

Hospitalized camels (n=30) were further divided based on their sex into females (n=13) and males (n=17). Hospitalized camels were admitted to veterinary clinics with different age ranged from 4 to 12 years old and were classified based on mean of age in to hospitalized camels < 6 and > 6 years old.

Clinical information

Camels' clinical history obtained from their owners. Physical examination, serum biochemistry and hemogram were determined for each camel. Body condition scoring in camels was done based on a visual and tactile evaluation of body fat reserves. BCS was evaluated by assigning a rating scale that ranges from 1 (indistinct hump on the dorsal length of the body, the ribs were markedly visible, the ischial and coxal tuberosities, transverse processes of vertebrae were very prominent and the rectogenital zone was very deep) to 5 (The hump base occupies more than 70% of the dorsal length of the body, the ribs were not visible, the ischial and coxal tuberosities, transverse processes

of vertebrae were not visible and not felt and the rectogenital zone was fully fat) (Crabb *et al.*, 1990; Faye *et al.*, 2001).

Sampling

Three blood samples were collected from jugular vein of each camel using sterile syringe. The first one was collected in dry clean labeled test tube contain anticoagulant, Ethylene diamine tetra acetic acid (EDTA) for complete blood count (CBC) and the second one contains heparin for determination of phagocytic index. While the third one 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 of a clear non-hemolysed serum for biochemical analysis. Sera were kept in deep freezer at -20 till biochemical analysis.

Hematological and biochemical measurements

Hematological examinations were carried according to method described by Schalm *et al.* (1975); Coles (1986) and Tietz (1999). Vitamin A (retinol) concentrations were measured chemically (1ml of each serum sample was mixed with 1.0 ml of 96.5% ethanol in a test tube, then added 3.5 ml 98.5% hexane. Tubes were shaken for 10 minutes and centrifuged at 800-× g for 10 minutes. The supernatants were measured at double waves length include 453 nm and 325 nm then final concentrations calculated by equations according to method described by Suzuki and Katoh (1990). Vitamin E (α-tocopherol) concentrations were assessed spectrophotometrically at different wave lengths with mathematical equations (Robert, 1964). Copper and zinc were measured in serum by UV- colorimetric spectrophotometric method (110-220 V UNICO Ltd, USA), using special copper and zinc kit produced by (Spectrum company, Egypt) according to method described by Johnsen and Eliasson (1987) and Abe *et al.* (1989). Selenium was measured in serum using acetylene air atomic absorption spectrophotometer (Tsoumbaris, 1990). Total protein and albumin were measured in serum by UV- colorimetric spectrophotometric method using total protein kit that was produced by Vitro Scient Company (Young, 2001). Globulin was calculated by subtraction of albumin value from total protein value and then A/G ratio was calculated by dividing albumin concentration on globulin concentration (Doumas, 1975).

Phagocytic activity and phagocytic index determination

Phagocytosis of polymorphnuclear cells using *Candida albicans* was performed according to the following method described by Wilkinson (1981).

Phagocytic activity % (PA) = Number of neutrophils ingesting *Candida albicans* / 100 neutrophil cells.

Phagocytic index (PI) = Total number of phagocytosed *Candida albicans* / Phagocytic activity.

Statistical analysis

Data were assessed for normality and they were normally distributed by Shapiro Wilk test. Statistical analysis was performed using the statistical package for social science (SPSS) for windows (Version 16.0; SPSS INC., Chicago, Ill.). Fisher's exact test was carried out to determine the proportions of vitamin A, E, copper, zinc and selenium concentrations in hospitalized camels. T test was used to compare between the two groups of camels, while MANOVA test was used to determine significant differences among subgroups of hospitalized camels with Duncan as a post hoc. Pearson's correlations were used to determine significance between phagocytic index and low concentrations of vitamin A, E, copper, zinc and selenium in hospitalized camels. Results were expressed as the mean \pm standard error (SEM). Odds ratios with 95% confidence interval (CI) were carried out to determine the effect of sex and age on the prevalence of the measured variables. Significance was set at $P < 0.05$. Cut-off values of vitamin A, E, zinc, selenium and copper concentrations were defined based on 5-95% CI of mean from apparently healthy camels where concentrations $< 5\%$ CI were considered low, while above 95% CI were defined as high concentrations.

Results

Prevalence of serum vitamin A, E and copper, zinc and selenium concentrations in hospitalized camels

In hospitalized camels, the prevalence of reduced vitamin A concentrations were more frequent than normal and elevated vitamin A concentrations in female hospitalized camels and age < 6 years old ($P < 0.05$; Table 1). Reduced vitamin E concentrations were more prevalent than normal and increased vitamin E concentrations in hospitalized female camels and camels with age < 6 and > 6 years old ($P < 0.05$; Table 1). Normocupraemia was more prevalent than reduced and elevated copper concentrations in hospitalized female and male camels, however, in hospitalized camels with age < 6 years, reduced and normal copper concentrations was more frequent than elevated copper concentrations ($P < 0.05$; Table 1). Low zinc concentrations were more frequent in female hospitalized camels and camels with age < 6 and > 6 years than normal and higher zinc concentrations ($P < 0.05$; Table 1). Low selenium concentrations were more frequent in females and males hospitalized camels than normal and high selenium concentrations ($P < 0.05$; Table 1).

Serum vitamin A and E, copper, zinc and selenium in healthy and hospitalized camels

Hospitalized female camels had lower serum concentrations of vitamin A and E, zinc and selenium compared to healthy and hospitalized male camels ($P < 0.05$; Table 2). Hospitalized male camels had low

concentrations of vitamin E, selenium and zinc compared to healthy ones ($P < 0.05$; Table 2). Hospitalized camels with age < 6 years old had lower serum concentrations of vitamin A and E, zinc and selenium compared to healthy and hospitalized camels of age > 6 years old ($P < 0.05$; Table 2). However, serum copper were not changed between healthy and hospitalized camels ($P > 0.05$) (Table 2). Serum vitamin A and E, zinc and selenium concentrations were lower in poor conditioned hospitalized camels compared to healthy ones ($P < 0.05$; Table 3).

Hematological examination in healthy and hospitalized camels

The mean values of the RBCs, Hb and PCV were significantly lower in hospitalized camels compared to apparently healthy ones ($P < 0.05$). Furthermore, RBCs, Hb and PCV were significantly lowered in subgroup 1 and subgroup 3 compared to other subgroups and apparently healthy ones ($P < 0.05$). MCV values were significantly higher in subgroups 1, 2, 3, and subgroup 5 compared to apparently healthy camels ($P < 0.05$), while no changes in values of MCH and MCHC between two main groups except subgroup 4 where MCHC value was significantly higher than control group as shown in Table 4. Table 4 illustrated that WBCs were significantly lower in hospitalized dromedary camels compared to apparently healthy ones ($P < 0.05$). Moreover, WBCs were significantly decreased in subgroups 1, 2 and subgroup 3 compared to other subgroups and healthy control ones ($P < 0.05$). The mean values of neutrophils were significantly lower in 5 subgroups compared healthy ones ($P < 0.05$). Lymphocytes were significantly lower in subgroup 1 and subgroup 3, but higher in subgroups 2, 4 and 5 compared to apparently healthy camels ($P < 0.05$). Eosinophils' count were statistically higher in subgroup 1 and subgroup 3 compared to other subgroups and healthy ones ($P < 0.05$). There is no changes in monocytes and basophiles between the two main groups and other subgroups ($P > 0.05$).

Total protein, Albumin, Globulin, and A/G ratio in healthy and hospitalized camels

Total protein and albumin concentrations were significantly lower in hospitalized dromedary camels compared to apparently healthy ones ($P < 0.05$). There is no significant changes in the mean values of globulin and A/G ratio between two main groups ($P > 0.05$) as demonstrated in Table 4.

Correlation between trace elements, vitamins and phagocytic index

Vitamin A concentrations were not correlated with phagocytic index in diseased dromedary camels, while there were a positively correlation between low concentration of serum copper, Selenium, and vitamin E and phagocytic index in diseased dromedary camels as documented in Table 5.

Table 1. Prevalence of serum vitamin A, E and copper, zinc and selenium concentrations in hospitalized camels.

Prevalence	Hospitalized (n=30)			
	Females (n=13)	Males (n=17)	< 6 years (n=19)	> 6 years (n=11)
Low vitamin A concentrations	61.5% (8/13)**	58.9% (10/17)	68.4% (13/19)**	54.5% (6/11)
Normal vitamin A concentration	38.5% (5/13)	41.1 (7/17)	31.6% (6/19)	45.6% (5/11)
Elevated vitamin A concentration	0	0	0	0
Low vitamin E concentrations	69.2% (9/13)*	52.9% (9/17)	73.6% (14/19)**	63.6% (7/11)**
Normal vitamin E concentration	30.8 (4/13)	47.1% (8/17)	26.4% (5/19)	36.4% (4/11)
Elevated vitamin E concentration	0	0	0	0
Low copper concentrations	23.1% (3/13)	41.2% (7/17)	42.1 (8/19)**	36.3 (4/11)**
Normal copper concentration	53.8% (7/13)**	52.9% (9/17)**	42.1% (8/19)**	54.6 (6/11)**
Elevated copper concentration	23.1% (3/13)	5.9% (1/17)	15.8% (3/19)	9.1% (1/11)
Low zinc concentrations	61.5 % (8/13)*	47.1 (8/17)	63.2% (12/19)**	72.7% (8/11)**
Normal Zinc concentrations	38.3 (5/13)	52.9 (9/17)	36.8% (7/19)	27.3% (3/11)
High Zinc concentrations	0	0	0	0
Low selenium concentrations	61.5% (8/13)**	76.5% (13/17)**	63.2 % (12/19)	45.5% (5/11)
Normal selenium concentrations	38.5% (5/13)	23.5% (4/17)	36.8% (7/19)	54.5% (6/11)
High selenium concentrations	0	0	0	0

(n): number; (*): P<0.05; (**): P<0.01.

Table 2. Serum Vitamin A and Vitamin E values in apparently healthy and hospitalized dromedary camels

Variables	Healthy Camels (n=65)	Hospitalized camels (30)			
		Females (n=13)	Males (n=17)	< 6 years (n=19)	> 6years (n=11)
Vitamin A (µg/dl)	69.1±2.3	60.5±1.2*	64.3±3.2	61.4±2.4*	64.6±0.9
Vitamin E (mg/100ml)	1.06±0.2	0.74±0.4**	0.84±0.1*	0.86±0.2*	0.93 ±0.1
Copper (µg/dl)	92.7±2.5	92.97±1.8	92.34±3.2	92.57±4.1	92.47 ±3.2
Selenium (ppm)	0.5±0.3	0.30±0.1*	0.36±0.2*	0.34±0.3*	0.45 ±0.2
Zinc (µg/dl)	121.2±4.6	104.2±5.2*	116.2±3.5	107.6±1.4*	114.8± 2.5

(n): number; (*): P<0.05; (**): P<0.01.

Table 3. Serum Vitamin A, Vitamin E and copper, selenium and zinc values in apparently healthy and hospitalized camels.

Variables	Healthy camels	Hospitalized camels
Vitamin A (µg/dl)	69.1±2.3	59.2±0.4*
Vitamin E (mg/100ml)	1.06±0.2	0.76±0.033*
Copper(µg/dl)	92.7±2.5	92.97±0.471
Selenium (ppm)	0.5±0.3	0.295±0.013**
Zinc(µg/dl)	121.2±4.6	104.2±2.4*

(*): P<0.05; (**): P<0.01.

Phagocytic activity and phagocytic index in healthy and hospitalized camels

The phagocytic activity and phagocytic index were significantly lower in hospitalized one dromedary camels compared to apparently healthy ones (P<0.05). The mean values of phagocytic activity were significantly lower in subgroups 3, 4 and subgroup 5 compared to other subgroups, while the mean values of phagocytic index were significantly decreased in subgroups 1, 3, and subgroup 5 as shown in Fig 1. Engulfed *Candida albicans* were presented in large

numbers by active neutrophils in good conditioned healthy camels (Fig 2 A). Blood film showed that large numbers of inactive neutrophils in hospitalized camels with low concentrations of vitamin A and E and trace elements (Fig 2B).

Serum vitamin A and E, copper, zinc and selenium concentrations in hospitalized camels based on sex and age

Female hospitalized camels were more likely to have low vitamin A concentrations compared to male hospitalized camels (Table 6). Hospitalized camels with age < 6 years old were more likely to have low vitamin A concentrations compared with hospitalized camels with age > 6 years old (Table 6). The likelihood of vitamin E deficiency was more significant in female hospitalized camels and younger age hospitalized camels < 6 years old (P<0.05; Table 6). The likelihood of copper concentrations were not significant between sex and age of hospitalized camels (P>0.05; Table 6). Female hospitalized camels were more likely to have low zinc and selenium concentrations compared with male hospitalized camels (Table 6).

Table 4. Hematological and biochemical analysis in apparently healthy camels, hospitalized dromedary camels and subgroups. Values were expressed as (MV±SE).

Variables	Apparently healthy camels (n=65)	Hospitalized camels (n=30)				
		Subgroup2.1 (↓Cu, Se) (n=10)	Subgroup2.2 (↓Cu, Vit A) (n=4)	Subgroup2.3 (↓Cu, Zn, Vit E) (n=7)	Subgroup2.4 (↓Vit E, Zn) (n=4)	Subgroup2.5 (↓Vit A, Se) (n=5)
RBCs (10 ⁶ /μl)	7.358±0.1071	5.56±0.04** ^a	6.15±0.15*	5.91±0.15** ^b	6.87±0.11*	6.18±0.28*
Hb (g/dl)	12.99±0.0859	10.2±0.05** ^a	11.2±0.14* ^b	11.1±0.38** ^c	12.8±0.14	11.6±0.26*
PCV (%)	29.33±0.3281	23.6±0.4** ^a	25.2±0.25** ^b	25.5±0.86** ^c	27.75±0.25*	26.2±0.31*
MCV (fl)	40.64±0.8006	42.4±0.4*	41.2±1.02*	43.05±0.9*	40.3±0.9	42±1.4*
MCH (pg)	17.79±0.3215	18.4±0.13	18.25±0.39	18.6±0.3	18.65±0.4	19.2±0.4
MCHC (%)	44.57±0.5103	43.4±0.7	43.2±1.03	43.1±0.9	46.08±0.4*	44.56±0.6
WBCs (10 ³ /μl)	13.84±0.156	10.7±0.1** ^a	11.6±0.08** ^b	11.5±0.3** ^c	12.35±0.22*	11.96±0.23*
Neutrophil (%)	42.44±0.143	41.3±0.21*	41.75±0.25*	41.29±0.18*	41.25±0.47*	41.2±0.48*
Lymphocyte (%)	51.00±0.190	50.2±0.13*	51.75±0.25	50.14±0.14*	52.25±0.25	52.4±0.244
Monocyte (%)	4.750±0.090	5.3±0.152	5±0.00	5.14±0.14	5±0.00	5±0.00
Esinophil (%)	1.327±0.085	2.6±0.16*	1±0.00	2.57±0.297*	1.25±0.25	1.2±0.2
Basophil (%)	0.5000±0.80	0.8±0.13	0.5±0.288	0.85±0.14	0.75±0.25	0.2±0.2
Total protein (gm/dl)	6.831±0.03558	6.06±0.065*	5.8±0.067*	6.025±0.08*	5.98±0.25*	5.99±0.11*
Albumin (gm/dl)	3.697±0.03962	3.014±0.04*	2.98±0.08*	2.95±0.04*	3.04±0.069*	3.12±0.107*
Globulin (gm/dl)	3.148±0.05249	3.046±0.06	3.03±0.08	3.07±0.066	3.04±0.069	3.12±0.107
A/G ratios	1.187±0.02844	0.958±0.032*	0.97±0.6	0.93±0.04*	1.02±0.04	0.96±0.057

(RBCs): red blood cells; (Hb): hemoglobin; (PCV): packed cell volume; (MCV): mean corpuscular volume; (MCH): mean corpuscular hemoglobin; (MCHC): mean corpuscular hemoglobin concentrations; (WBCs): white blood cells; (A/G): Albumin/Globulin, (Cu): copper; (Se): selenium; (Zn): zinc; (Vit A): vitamin A; (Vit E): vitamin E. (*): P<0.05; (**): P<0.01. Subgroups with different letters (^{a, b, c}) indicate statistical significantly difference than other subgroups (P<0.05).

Table 5. Pearson correlation (r) between low concentration of copper, zinc, selenium, vitamin A, vitamin E and phagocytic index in diseased dromedary camels.

Variables	Phagocytic index (r)	P-value
Copper	0.5	0.03**
Zinc	0.07	0.7
Selenium	0.490	0.02*
Vitamin A	0.03	0.6
Vitamin E	0.5	0.02**

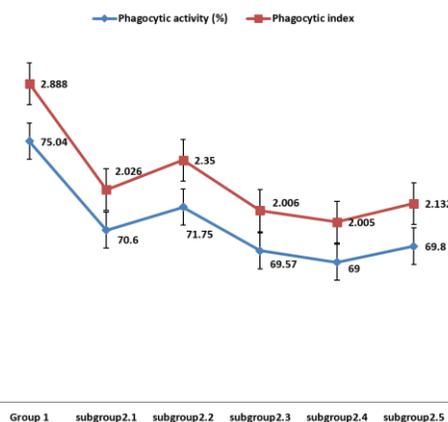


Fig. 1. Phagocytic activity and phagocytic index in apparently healthy and hospitalized dromedary camels. Values were expressed (Mean ± SE).

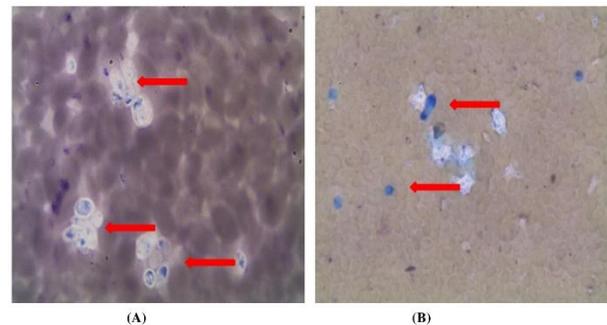


Fig 2. (A): Blood film showed that engulfed *Candida albicans* (large numbers) by active neutrophils in good conditioned camel (red arrows). **(B):** Blood film showed that large numbers of inactive neutrophils in hospitalized camels (red arrows).

Discussion

In the study reported here, erythrogram and leucogram, serum vitamin A and E concentrations were significantly lower in hospitalized camels compared to healthy ones. Furthermore, trace elements concentrations including copper, zinc and selenium were also lower in hospitalized camels than healthy camels. Of interest, serum vitamin A and E concentrations in addition to serum copper, zinc and selenium concentrations were positively associated with hospitalized camels' immune status.

Table 6. Univariate analysis of vitamins and trace elements in hospitalized camels based on sex and age.

Low vitamin A concentrations < 64.3 µg/dl		OR	95% CI	P-Value
Sex	Female	2.1*	1.04-5.2	0.03
	Male	0.85	0.65-3.42	0.6
Age	< 6 years	1.4*	1.02-2.6	0.04
	> 6 years	1.2	0.82-4.9	0.4
Low vitamin E concentrations < 0.8 (100 mg/ml)		OR	95% CI	P-Value
Sex	Female	1.7*	1.22-3.8	0.04
	Male	0.63	0.56-1.84	0.8
Age	< 6 years	1.32*	1.12-4.2	0.03
	> 6 years	0.75	0.45-1.75	0.6

Additionally the likelihood of low concentrations of vitamin A, E, zinc and selenium concentrations were associated with female hospitalized camels and younger ones < 6 years old during time of admission. RBCS, Hb and PCV values were significantly lower in hospitalized camels compared to healthy ones, similar results were reported by previous camel research studies (Gernsten, 2009; Bunn, 2011; DeMoranville and Best, 2013). The lowest values of RBCs, Hb and PCV were recorded in subgroup 1 and subgroup 3 where copper was deficient between two subgroups. Potential explanations are multifactorial including malnutrition, iron, copper, folate, vitamin B12, vitamin B6 deficiencies (Gernsten, 2009; Bunn, 2011). However, folate, vitamin B6 and B12 concentrations were not measured in this study. Another possible explanation is reduced concentrations of vitamin A and selenium in hospitalized camels (Faye *et al.*, 2009). It was documented that vitamin A and selenium deficiency was associated with remarkable signs of anemia and reduction of hemoglobin concentrations, PCV and RBCS comparing to apparently healthy camels (El Khouly *et al.*, 2001; Faye *et al.*, 2009). Thereby, it is feasible that reduced concentrations of vitamin A, E, selenium and copper due to the effect of diseases leads to alteration of erythrogram dynamics in camels. The hospitalized camels of this study have shown clinical signs of diarrhea and/or constipation suggesting digestive disorders (Alsaad, 2009). Furthermore, the presence of ticks and pale mucus membrane of hospitalized camels could indicate that increased prevalence of blood parasite in the reared area of camels that in turn could play a role in alteration of erythrogram and immune status of hospitalized camels (Alsaad, 2009).

Interestingly, WBCS were significantly lower in hospitalized camels. Furthermore, we documented neutropenia, lymphopenia, eosinophilia in hospitalized camels. Decreased neutrophil and lymphocytic count in hospitalized camels could be attributed to decreased copper and zinc concentrations which in turn suppress

immunomodulation (Niederman *et al.*, 1994; Nockels, 1994; Soetan *et al.*, 2013). However, copper concentrations was within normal range in poor conditioned diseased camels suggesting that copper may not play a role in decreasing WBCS values in hospitalized dromedary camels. It is plausible that parasitic infestation stimulate eosinophils production in hospitalized camels (Padmaja, 2012).

We have shown that hospitalized camels had reduced concentrations of vitamin A compared to healthy ones. The reduced concentrations of vitamin A in hospitalized camels may be attributed to destruction of vitamin A in diet by many factors such as oxygen, heat, light and acids and/or liver diseases (Olson, 1990; Baraka, 2012). Moreover, we did not find association with its low concentrations with camels' phagocytic activity and index indicating that vitamin A may not play a direct role in camel's immune status..

Serum vitamin E concentrations were significantly lower in hospitalized camels than healthy camels. Possible explanations include prolonged storage of animal hay for long time or secondary to decreased supplementation of vitamin E in camels' ration (Seboussi *et al.*, 2008; Baraka, 2012). In this study, there was a significant medium positive correlation between vitamin E and phagocytic index suggesting its role in stimulation of immune system against infections as anti-oxidant in hospitalized camels. A literature has been reported that vitamin E scavenges free radicals e.g. peroxy radicals and converted into tocopherol radicals, while at the level of trace elements, they can act as pro-oxidants and stimulate leukocytes and macrophages during phagocytosis (McDowell *et al.*, 2007).

Serum trace elements concentrations were lower in hospitalized camels than healthy ones except copper where its concentration was not statistically different between both groups of camels. However, another study documented that the decreased concentrations of copper in hospitalized camels could be attributed to feeding on sandy soil and ration rich in molybdenum

and sulphates that interfere with copper absorption (Amy, 2011). Other factors that could contribute in reduced selenium and zinc concentrations in hospitalized camels include stress factors, unsaturated fatty acids in clover pastures and vitamin E deficiency (Graham, 1991).

Our study was also showed a medium significant association between low copper concentrations and phagocytic activity and index in hospitalized camels. However, selenium was positively correlated with phagocytic index and activity in hospitalized camels, this correlation remain weak in this study. It has been reported that trace elements are essential for immunomodulation through stimulation of phagocytic cells proliferation and migrations (Hefnawy and Tortora, 2010). Additionally a recent literature stated that copper, zinc and selenium supplementation improve phagocytic activity and copper/zinc-superoxide dismutase and ceruloplasmin in animals (Yatoo *et al.*, 2013) suggesting that low concentrations of trace elements may play a role in decreasing resistance of dromedary camels against pathological conditions.

Concentrations of total protein and albumin were decreased in hospitalized camels compared to apparently healthy ones. Potential explanations include digestive disorders such as liver affections and decrease protein levels during blood parasite course (Alsaad, 2009). However, liver biomarkers were not assessed in the present study.

In young and female hospitalized camels, serum vitamin A and E, zinc and selenium concentrations were more likely to be low compared to male and old age hospitalized ones. We speculate oxidative stress levels were higher in female hospitalized camels and camels with age below than 6 years old that in turn could decrease concentrations of vitamin A, E, zinc and selenium. Similar result was observed in human patients (Brunelli *et al.*, 2014). Reduced selenium concentrations were common in young animals and lead to white muscle disease including degenerative myocarditis and discoloration of skeletal muscle (Faye and Seboussi, 2009). However, oxidative biomarkers were not measured that considered a limitation of this study.

Conclusions

In conclusion, reduced vitamin A and E and other trace elements are frequent in hospitalized dromedary camels during course of diseases. Vitamin E, selenium and zinc concentrations could be used as predictors of health outcomes in hospitalized camels because of their association with phagocytic activity and index allowing hospitalized camels less resistant to the diseases. Low vitamin A and E together with decreased zinc and selenium concentrations in female and young age hospitalized camels at time of admission may indicate

higher oxidative stress. However, future studies will be required to assess oxidative biomarkers based on different age and sex to monitor diseases progression in hospitalized camels. This study was carried out on small number of hospitalized camels (n=30) at the veterinary hospital which is considered a limitation, but the information from this study could be a predictor of health outcomes in hospitalized dromedary camels.

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Conflict of interest

The authors declare that there is no conflict of interest.

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