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ASSOCIATION OF VITAMIN B12, COBALT AND SULFUR LEVELS IN SERUM AND CEREBROSPINAL FLUID OF DROMEDARY CAMELS WITH NEUROLOGICAL SIGNS

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ABSTRACT

The hypothesis of this study was that higher levels of sulfur following chronic digestive disorders in camels could decrease the concentration of cobalt and vitamin B12, which possibly might cause neurological disorders. Therefore, this study was aimed to determine levels of vitamin B12, cobalt and sulfur in serum and CSF of camels with neurological signs. Five apparently healthy camels and 7 dromedary camels with neurological signs like shivering, tremor, staggering, rotation of the head, slight vision impairment and progressive worsening general condition were included in the present study. The diseased animals showed previously chronic digestive problems like constipation, emaciation and weakness. Clinical examination and collection of blood and cerebrospinal fluid samples were done. The concentrations of vitamins B12, cobalt and sulfur in cerebrospinal fluid and serum samples were determined using HPLC assay. There was a decrease in levels of vitamin B12 and cobalt in serum and CSF for affected camels, while there was an increase in the level of sulfur in serum and CSF of affected camels as compared to healthy. The obtained results of serum and CSF in healthy and diseased animals could help in early diagnosis of neurological disorders in camel.

Key words: Camel, cobalt, CSF, neurological, serum, sulfur, vitamin

Neurological signs in camels can be categorised according to its origin into infectious or noninfectious causes (Baaissa *et al,* 2018; Shoeib *et al,* 2019). The neurological signs in camels can be characterised by behavioural and neurological changes, meningitis, encephalitis, meningoencephalitis, stillbirth and abortion (El Dobab *et al,* 2008). Vitamin B12 (cobalamin), which is synthesised in ruminants by ruminal flora (Mohamed, 2006), is closely associated with neurological functions (Nijst *et al,* 1990). Cobalt plays an essential role for ruminal synthesis of vitamin B12 as Co resides at the centre of the circle of vitamin B12 (McDowell, 2000). There are no clinical disorders reported in the literature due to deficiency of cobalt in the diet (Faye and Bengoumi, 2018). Determination of serum or plasma cobalt concentration in dromedary camel is very rare. However, researchers (Deen *et al,* 2004; Shen and X, 2010) studied the levels of cobalt on Bactrian camel. It found in ruminants also that the toxicity of sulfur is mostly related to an increased sulfide production due to the microbial reduction of sulfate in the rumen, which could cause diarrhoea, respiratory and nervous symptoms (Alves de Oliveira *et al,* 1996). However, Hooshmand *et al* (2016) reported a close connection between vitamin B12 and sulfur, which could cause the decrease concentration of vitamin B12 with increased concentration of sulfur neurological disorders (Stabler, 2013). Clinical observation of many camels with neurological symptoms in the eastern region of the Saudi Arabia revealed that they had suffered previously from digestive problems like chronic constipation or that they were in an environment in which the soil and water contained high concentrations of sulfur. However, El Dobab *et al* (2008) observed a high neurological injury in camels consuming water containing high levels of sulfur. Changes in the cerebrospinal fluid composition may reflect central nervous system (CNS) injuries and many pathological processes, because this fluid originates from CNS structures (Lardinois *et al,* 2015). Cerebrospinal fluid analysis forms the essential diagnostic evaluation of ruminants with clinical symptoms involving the central nervous system (Camara *et al,* 2020). Recently, Shawaf *et al* (2020) reported some values of CSF

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constituents from healthy and affected camels with neurological disorders in Saudi Arabia. Although the debate of valuableness of cerebrospinal fluid analysis in the diagnosis of neurological disorders, serum, cerebrospinal fluid and their comparisons may provide a wide range of valuable biochemical and cellular information that help in evaluation of nervous system health of animals (AI-Sagair *et al,* 2005; Camara *et al,* 2020; Welles *et al,* 1992). The hypotheses of the study were the higher levels of sulfur after digestive disorders in camels could cause later decrease concentration of cobalt and vitamin B12, which could cause neurological disorders. Therefore, this study was aimed to determine levels of vitamin B12, cobalt and sulfur in serum and CSF of camels with neurological manifestations and correlate these finding with diagnosis of these cases.

Materials and Methods

In this study, seven diseased dromedary camels (aging 5-14 years) presented to Veterinary Teaching Hospital, King Faisal University were included and these camels showed neurological signs with previous history of chronic digestive problems causing constipation, emaciation and weakness. The predominant neurological signs observed were shivering, tremor, staggering, rotation of the head, slight vision impairment and progressive worsening of general condition. Five apparently healthy camels were used for comparison. All animals were examined clinically and blood samples were obtained from the jugular vein. CSF samples were taken from the atlanto-occipital articulation under sedation (Shawaf *et al,* 2018).

Vitamin B12 analysis

The concentrations of Vitamins B12 in cerebrospinal fluid and serum samples were determined using HPLC assay. Vitamins B12 were purchased from ACROS ORGANICS (New Jersey, USA, 1-800-ACROS-01, Geel, Belgium). Cyanocobalamin (B12, 96% extra pure, HPLC assay, lot: A0304024, code: 405920010). Ethyl acetate, water, ammonium acetate and methanol HPLC grade were purchased from Sigma Aldrich (USA). Serum samples (0.250 mL), 25 µL NaOH (0.5 mM) and 25 µL IS (1µg mL^{-1}) were vortex-mixed for 0.5 min, then 2 mL of ethyl acetate was added and vortexed again for 2 min, followed by centrifugation at 5000 rpm for 6 min at 4°C. The organic layer was separated and transferred into another polypropylene tube, and evaporated to dryness at 37°C under gentle flow of nitrogen gas. The residue was reconstituted with 100 µL mobile phase, and 7 µL was injected for analysis. UPLC/ESI–MS/MS analysis was done according to (Geng *et al,* 2017). An ultra-performance liquid chromatography (UPLCTM) system Acquity (Waters, Mildford, MA, USA) was interfaced to a triple quadruple mass spectrometer (UPLC/MSMS) (TQDTM, Waters Micro mass, Manchester, UK) using an electrospray interface. Vitamins B12 of serum and CSF were separated, using an Acquity UPLC BEH C18 analytical column, 1.7 µm particle size, 2.1mm × 50 mm (Waters). The column was eluted with the mobile phase of methanol: ammonium acetate 5 mM (60:40, v/v) at a flow rate of 0.3 mL min^{-1} with column oven 30°C. Calibration curves were prepared for control and quantification purposes according to Geng *et al* (2017). Serum and CSF samples extracts (after reconstitution in mobile phase) were spiked with different aliquots of Vitamins B12 standard solution to give final concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 ng/ml.

Cobalt analysis

Concentrations of cobalt were estimated in serum and CSF samples by using AA-7000 Shimadzu (Koyoto, Japan) atomic absorption spectrophotometer coupled with a FAAS Flame Atomic Absorption, GFA-7000 graphite furnace atomizer, and ASC-7000 auto sampler from Shimadzu (Koyoto, Japan) was used. A high-density graphite tube was used for atomisation (Meligy, 2018). The digestion procedures were done by using the microwave method Usero *et al* (2005) by using the Microwave digestion system Model MARSXpress 907511 (CEM Cooperation, Mathews, North Carolina, USA) according to USEPA method 3051. 0.5 gm of each serum samples were placed in [polytetrafluoroethylene (PTFE)] digestion vessels with 6mL of nitric acid (65%) and 2mL of hydrogen peroxide (30%).The samples in the vessels were then digested using an optimised microwave method as described (Meligy, 2018). The contents of the tubes were cooled then diluted to 50 mL with De-ionised doubly distilled water (DDDW).

Sulfur analysis

Concentrations of Sulfur were estimated in serum and CSF samples by using high-resolution continuum source atomic and molecular absorption spectrometer (Analytik Jena, Jena, Germany) (Andrade-Carpente *et al,* 2016). The digestion procedures were done by using the Microwave digestion system Model MARSXpress 907511 (CEM Cooperation, Mathews, North Carolina, USA) according to USEPA method 3051 (Usero *et al,* 2005). Each serum sample (0.5

gm) was placed in [polytetrafluoroethylene (PTFE)] digestion vessels with 6mL of nitric acid (65%) and 2mL of hydrogen peroxide (30%). The samples in the vessels were then digested using an optimised microwave method (Meligy, 2018).

Statistical analysis

Statistical analysis was performed after the data has been recorded in Excel spreadsheets and imported into Stata version 14 (Stata Corp., TX, USA) using the GraphPad Prism (v. 5) software. Results were expressed as means ± S.E. of the mean (SEM). Student's t test was used for difference analysis between means. Variation within each parameter was evaluated using coefficient of variation (CV). Effects were considered statistically significant at p value of less than 0.05.

Results and Discussion

Table 1 and figs 1, 2 and 3 showed the serum and CSF levels of vitamin B1, sulfur and cobalt in healthy and camels with neurological signs, respectively. Vitamin B12 levels for serum and CSF of affected camel were 27.3±0.94 ng/dL and 10.3±0.47 ng/dL, respectively which were lower as compared to healthy camels where these were 46.79±1.77 ng/dL; 16.21±1.09 ng/dL, respectively. On the other hand, there were higher concentration of vitamin B12 in serum for healthy and diseased camels as compared to their values in CSF. However, vitamin B12 deficiency was certainly not clinically reported in camel (Faye and Bengoumi, 2018). Lower results for B12 in serum of healthy camel was reported in camel (Mohamed, 2006) and in sheep (Clark *et al,* 1989) compared to its levels in the present study. However, Kather *et al* (2020) reported similar results for vitamin B12 in serum of healthy dogs. CSF vitamin B12 could deliver significant additional evidence to understand some neurological disorders in human and animals (Gianazza *et al,* 2003). In agreement to the present study Christine *et al* (2020) stated a closer relationship between the concentrations of vitamin B12 in serum and CSF. Lower levels for vitamin B12 in CSF of healthy camels in the present study was reported previously in CSF of healthy people (Nijst *et al,* 1990). Christine *et al* (2020) also reported similar results for levels of vitamin B12 in CSF of people affected with neurological disorders. The decreased levels of vitamin B12 in serum and CSF for diseased animals compared to healthy in the present study could be explained by considering that the diseased camels showed neurological symptoms with a previous history of chronic digestive disorders causing emaciation and weakness, which may cause a decreased vitamin B12 levels in the serum and CSF (Friesecke, 1980). The difference values for B12 in serum and CSF of healthy and affected camels in the present study may be due to several factors, including the pathogenesis of the neurological diseases in camels, which are still not well studied (El Dobab *et al,* 2008).

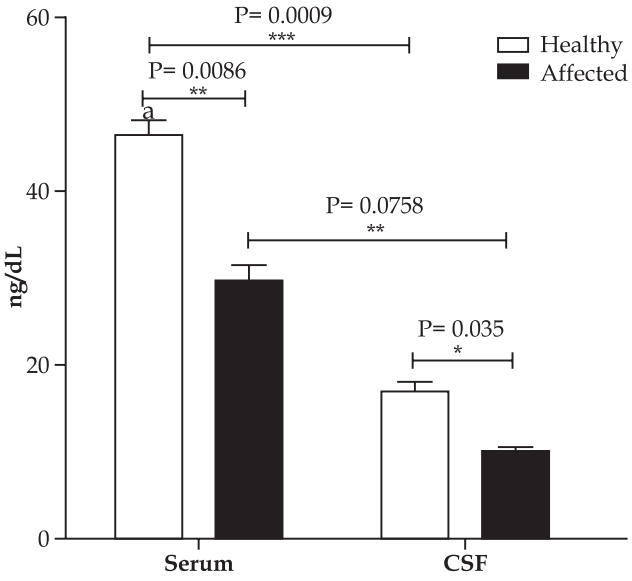

Fig 1. Vitamin B12 levels (ng/dL) in serum and cerebrospinal fluid (CSF) of healthy and camel with neurological signs. *<0.05, (01, ***<0.001.

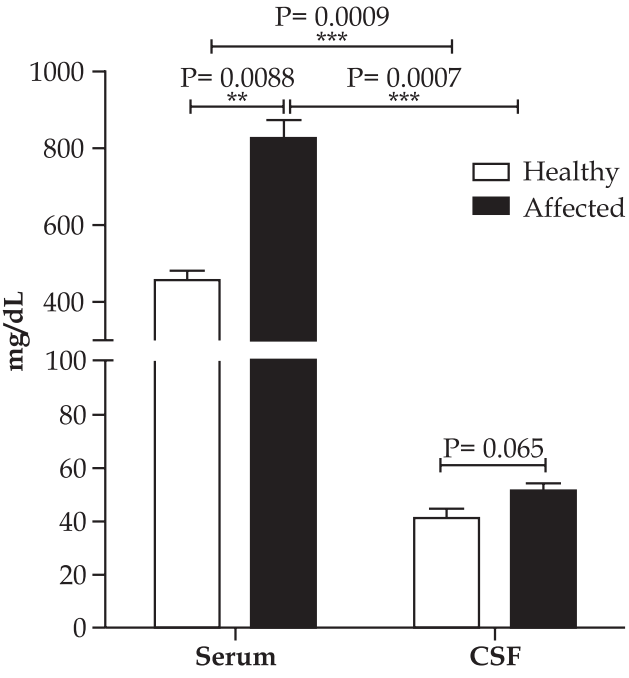

Fig 2. The concentration of sulfur (mg/dL) in serum and cerebrospinal fluid in healthy and camel with neurological signs. NS>0.05, **<0.01, ***<0.001.

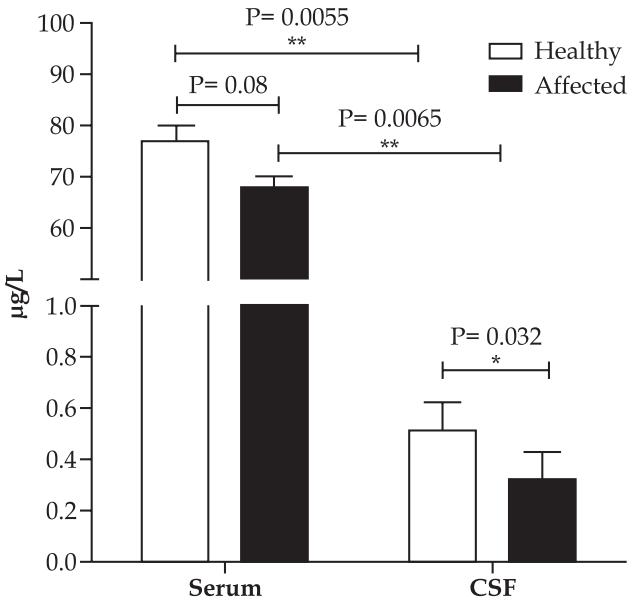

Fig 3. Cobalt levels (µg/L) in serum and cerebrospinal fluid in healthy and camel with neurological signs (NS>0.05, *<0.05, **<0.01).

Cobalt is an essential mineral to mammals in the form of methyl cobalamin (Roos *et al,* 2013). A closer relationship between vitamin B12 and cobalt is important in ruminants. Cobalt is a part of the molecule and essential to its synthesis in the rumen, its deficiency can result in vitamin B12 deficiency (Faye and Bengoumi, 2018). In the present study, there was no significant difference in serum of cobalt levels among healthy (77.18±2.68 µg/L) and diseased (67.56±2.54 µg/L) camels, while the cobalt levels in CSF for affected camel (0.32±0.1 µg/L) was lower than in healthy camels (0.51±0.11 µg/L). In agreement with the present study, Burenbayar (1989) reported similar levels for cobalt in serum of healthy camels, while Shen and Li (2010) and Zongping (2003) reported higher levels for cobalt in serum of healthy Bactrian camels. Similar results for decreased cobalt in diseased sheep was reported by MacPherson *et al* (1976), who stated that the animals with cobalt deficiency showed cerebrocortical necrosis. Sanyal *et al* (2016) reported decreased levels in serum cobalt in people with neurological disorders. The ratio of cobalt concentration in CSF/serum in healthy camels was about 1% in present study, while Stojsavljevic *et al* (2020) reported 10% ratio in human. Contrary to the results of present study, Sanyal *et al* (2016) found equal levels for cobalt in serum and CSF for healthy people. Similar results for decreased levels of cobalt in CSF in diseased camels were reported in people with neurological disorders (Sanyal *et al,* 2016).

In ruminants, it is evident that a diet or drinking water containing high levels of sulfur caused neurological disorders (Alswailem *et al,* 2009; Niles *et al,* 2000) resulting from brain malacia (Rousseaux *et al,* 1991). Sulfur levels in the present study for serum of affected camels (824.6±47.91 mg/dL) was higher as compared to its levels in serum of healthy camels (456.9±23.7 mg/dL). In central Saudi Arabia, Alswailem *et al* (2009) reported diseased camels with neurological disorders living in environment with high concentrations of sulfur in soil and water. The possible relationship between higher levels of sulfur in serum and CSF with neurological symptoms in diseased camels in this study could be explained by the fact that the animals with a previous digestive system disorders could cause the high sulfide production due to the microbial reduction of sulfate in the rumen (Alves de Oliveira *et al,* 1996). There were no significant changes for sulfur levels for CSF in healthy and affected animals (41.5±3.54 mg/dL; 51.87±2.84 mg/dL), respectively. Lower concentrations for sulfur in CSF was reported in healthy people (Gellein *et al,* 2008). In agreement with the results in this study for diseased camels, Gellein *et al* (2008) reported similar values for sulfur concentration in CSF in people affected with neurological disorders. However, Batista *et al* (2013) and Camara *et al* (2020) presented neurological

Table 1. The mean ± SEM with p values of B12, cobalt and sulfur levels in serum and CSF of healthy camels and those with neurological signs.

Parameter		Healthy (N=5)		Diseased (N=7)	
		Mean ± SEM	Range	Mean ± SEM	Range
Vitamin B12 (ng/dL)	Serum	46.79 ± 1.77	28-59.9	27.03 ± 0.94	18.6-35
	CSF	16.21 ± 1.09	9-27.3	10.03 ±0.47	5.9-13.2
Cobalt (µg/L)	Serum	77.18 ± 2.68	69.32-84.12	67.56 ± 2.54	60.14-75.48
	CSF	0.51 ± 0.11	0.12-0.9	0.32 ± 0.1	0.09-0.9
Sulfur (mg/dL)	Serum	456.9 ± 23.7	380-570	824.6 ± 47.91	712-1100
	CSF	41.5 ± 3.54	28.2-52.9	51.87 ± 2.84	38.8-60.3

disorders (Polioencephalomalacia) with significant changes in CSF of sheep and goats correlated with sulfur poisoning from water contaminated by petroleum. In the present study, higher levels for serum sulfur than that in CSF of healthy and affected camels were reported, which is in agreement with previous studies (Gellein *et al,* 2008).

In conclusion, higher levels of sulfur in serum and CSF could decrease the levels of vitamin B12 and cobalt in camels, which possibly precipitated neurological signs. Therefore, vitamin B12 and cobalt might prove logical in treatment of chronic digestive disorders in these animals. The obtained results provide reference values for serum and CSF vitamins B12, cobalt and sulfur levels for further studies and could assist in the diagnosis and treatments of camel neurological disorders.

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