Levels of Reduced Glutathione in Erythrocyte of different Arabian camel (*Camelus dromedaries)* Breeds

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Abstract: The maintenance of reduced glutathione (GSH) content in camel's erythrocytes is essential for their viability as this tripeptide protects the cell components from oxidative damage. The reduced glutathione level has been estimated in four different breeds of Arabian camel, The overall GSH level in camels was found as 6.7± 0.37 mmol/gHb, with the highest concentration in the Majaheem (8.6 ± 0.4 mmol/gHb), and lowest in Shaele (5.13 ± 0.3 mmol/gHb) breeds. The effect of breed was found to be a significant on glutathione level. Higher levels of GSH were found in mature adult as compared with immature and aged. Aged animals showed a higher level of GSH if compared with immature. Males of all breeds in all ages have a significant higher RBCs GSH concentration than females. in conclusion, the RBCs GSH concentrations were varied with in the Arabian camel breed, sex and age.
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Key wards: Reduced glutathione, *Camelus dromedaries*, age, sex, breed

1. Introduction

The tripeptide glutathione (GSH), comprised of the amino acids L-cysteine, glycine, and L-glutamate, is found in all cells of aerobic organisms and plays numerous, critical roles as an antioxidant and nucleophile in regulating cellular homeostasis and drug metabolism. GSH is synthesized exclusively in the cytoplasm of most cells by two ATP-dependent reactions. Despite this compartmentation, GSH is found in other subcellular compartments, including mitochondria (Lawrence, 2012). GSH is the principal endogenous non protein thiol component of the antioxidant defense system in living cells-(Abdurrahman, 2010). Glutathione has multiple functions, as a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species (ROS and RNS, and electrophiles or by operating as a cofactor for various enzymes. Glutathione is moderately stable in the intracellular milieus because intracellular peptidases can cleave peptide bonds formed by the α-carboxyl groups of amino acids, but typically not the γ-carboxyl groups (Volodymyr, 2012). Reed blood cell (RBC) membranes contain lipids rich in unsaturated fatty acids. RBCs are more frequently exposed to oxygen than other body tissue and, thus, are more susceptible to oxidative damage. Invasion of the RBC membrane by peroxidants may lead to cell hemolysis. Moreover, the hemoglobin in RBCs is a strong catalyst which may initiate lipid peroxidation.In addition to lipid peroxidation, oxidants affect vital -SH groups of proteins which are highly active and may be targeted during oxidative stress. Reduced glutathione levels lead to a decrease in -SH groups. Glutathione directly protects membrane proteins and preserves their stability. Decreased levels of glutathione result in oxidization of membrane -SH groups and loss of membrane stability, so comparison to other organs, erythrocytes are more susceptible to oxidative stress during their normal aerobic functions, GSH plays an essential role in the protection of erythrocyte hemoglobin, cell membranes, and other cell constituents from oxidative damage (Asgary *et al.,* 2005).

The Arabian camel (*Camelus dromedarius*) has the ability to withstand extremely dry climate and hot temperatures of the desert. The peculiar physiological and biochemical functions of camel erythrocytes play a principle role in adaptive mechanism to heat and severe dehydration. Camel erythrocytes are resistant to hemolysis compared with other species may be due to the high activity of Glucose-6-phosphate dehydrogenase (G6PD) in camel erythrocytes compared to that of human and other mammalian cells, which indicate higher generation of NADPH, possibly to fulfill a higher requirement for NADPH, necessary for generation of GSH from GSSG in presence of Glutathione reductase, (Abdurrahman, 2010).Thus the purpose of our study is to determine the normal reference ranges of the blood GSH levels in dromedaries, according to sex, age, and breed.

2. Material and methods

The study was conducted in the west region of Saudi Arabia, at December 2012. A total of 120 Arabian Camels of 4 breeds, Majaheem, Sofor, Shaele and Wodoh were used in this study, in each breed 15 female (5 under 4 years , 5 from 4 to 10 years and the other over 10 years) and 15 males (5 under 4 years , 5 from 4 to 10 years and the other over 10 years), all femals of 5-10 years group were non lactating pregnant and the other were nether pregnant nor lactating , all animals were apparently healthy and feed on fed Lucerne (Medicago sativa) and pelleted concentrate mixture (wheat bran 36%, corn 25.5%, cotton seed cake 35.5% calcium carbonate 2%, sodium chloride 1%). Water and food were provided ad-libitum.

Sample collection and preparation:

Blood samples were collected by venipuncture from the left jugular vein in heparanized tubes from all animals, transported in cooling condition to the Biotechnology Labe of Biological Science department, Faculty of Science, King Abdul Aziz University, North campus, Saudi Arabia. The blood was centrifuged at 900 g for 15 min at 5°C and plasma was harvested. Red blood cells were rinsed three times with isotonic solution of NaCl (0.9%) and centrifuged for 10 min at 3000 g. The supernatant was discarded and the red blood cells were processed. red cells were hemolysed with 9-fold volume distilled water to prepare 10% (v/v) hemolysate (Mousa *et al.,* 2006). Hemoglobin (Hb) concentration were estimated using a commercial kits supplied by spinreact company (Ref 1001231) following the manufacture instructions.

Determination of RBCs reduced glutathione:

GSH concentration in the RBCs hemolysate was measured using the method of Beutler et al. (1963); this method is based on the development of a stable yellow color when 2-nitrobenzoic acid is added to sulfhydryl compounds. The amount of reduced product, thionitrobenzene, was measured at 412 nm and expressed as mmol/g Hb.

Statistical analysis:

The data was processed using the statistical package for social science (SPSS Inc., Version 13, Chicago, Illinois, USA). All results are expressed as mean ± SD. Comparison among groups was made by one-way analysis of variance (ANOVA). Duncan's test was used for testing the inter-grouping homogeneity. Statistical significance was set $p< 0.05$.

3. Results

Reduced glutathione concentrations were significantly differ among the studded breeds of the same sex (table 1,2 and 3), except Sofor and Wodoh females under five years (table 1) as well as Sofor with Shaele and Sofor with Wodoh males over 4 and 10 years respectively (table 1 and 2). Majaheem camels–have the highest GSH concentration but Shaele has the lowest concentration in comparing to the other breeds of the same sex. In relation to the camel sex, males of all studded breeds have a higher GSH concentration than females of the same breed (table 1,2 and 3) except Sofor and Shaele under 4 years (table 1). GSH concentrations in the same breed and sex were affected by the camel's age although it was significantly increased in mature adult (from 4-10 years) if compared with young and aged camels, in the same time the aged camels (over 10 years) have higher concentrations than that of young camels (table 4 and 5).

Table 1. GSH concentrations M ± SD (mmol/gHb) in both females and males of the studded camel breeds under 5 years old.

Sex	Majaheem	Sofor	Shaele	Wodoh
Females	$5.5 \pm o.5^{b}$	4.4 ± 0.41^{c}	3.43 ± 0.4^{d}	4.4 ± 0.45^{c}
Males	6.5 ± 0.5^{a}	4.5 ± 0.5^{c}	4.03 ± 0.15^{cd}	5.21 ± 0.37^{b}

Means carrying different subscript are significant differ at $P < 0.05$

Table 2. GSH concentrations M ± SD (mmol/gHb) in both females and males of the studded camel breeds from 5 to 10 years old.

Sex	Majaheem	Sofor	Shaele	Wodoh
Females	10.5 ± 0.45^{b}	8.5 ± 0.5^{d}	6.5 ± 0.5^{f}	8 ± 0.1^{d}
Males	13.23 ± 0.25^{a}	10.1 ± 0.41^{bc}	7.2 ± 0.25^{e}	$9.5 \pm o.5^{c}$

Means carrying different subscript are significant differ at $P < 0.05$

Table 3. GSH concentrations M ± SD (mmol/gHb) in both females and males of the studded camel breeds over 10 years old.

Sex	Majaheem	Sofor	Shaele	Wodoh
Females	7.5 ± 0.4^{b}	5.46 ± 0.45^{d}	4.2 ± 0.25^{e}	5.5 ± 0.5^{d}
Males	8.2 ± 0.25^{a}	6.46 ± 0.45^{c}	5.4 ± 0.32^{d}	7.2 ± 0.25^{b}

Means carrying different subscript are significant differ at $P < 0.05$

Table 4. GSH concentrations M ± SD (mmol/gHb) in females of the studded camel breeds of different ages.

Age	Majaheem	Sofor	Shaele	Wodoh
Under 4 years	5.5 ± 0.5^{c}	4.46 ± 0.4^{c}	3.4 ± 0.4^{c}	4.4 ± 0.45^{c}
From 4- 10 years	10.5 ± 0.45^{a}	8.5 ± 0.5^{a}	6.5 ± 0.5^{a}	8 ± 0.1^{a}
Over 10 years	7.46 ± 0.45^{b}	5.5 ± 0.45^{b}	4.2 ± 0.25^{b}	5.5 ± 0.5^{b}

Means of the same column carrying different subscript are significant differ at $P < 0.05$

Table 5. GSH concentrations M ± SD (mmol/gHb) in males of the studded camel breeds of different ages.

Age	Majaheem	Sofor	Shaele	Wodoh
Under 4 years	6.5 ± 0.5^{c}	4.5 ± 0.5^{c}	4.03 ± 0.15^{c}	5.21 ± 0.37^{c}
From 4- 10 years	13.2 ± 0.25^{a}	10.13 ± 0.41^{a}	7.2 ± 0.25^{a}	9.5 ± 0.5^{a}
Over 10 years	8.2 ± 0.26^{b}	6.46 ± 0.45^{b}	5.4 ± 0.32^{b}	7.2 ± 0.25^{b}

Means of the same column carrying different subscript are significant differ at $P < 0.05$

4. Discussion

The Arabian camel (*Camelus dromedarius*) has adapted to withstand extremely stressful dry climate and hot temperatures of the desert, camel erythrocytes play a principle role in adaptive mechanism to heat and severe dehydration. Camel erythrocytes are resistant to hemolysis if compared with other species this may be due to the high activity of Glucose-6-phosphate dehydrogenase (G6PD) in camel erythrocytes compared to human and other mammalian cells, which indicate high generation of NADPH, possibly to fulfill the high requirement for NADPH, necessary for generation of GSH. Reduced glutathione was measured in man, guinea pig, sheep, rat, rabbit, cattle and dromedary. Reduced glutathione was present in all mentioned species but at different concentrations. If species are placed in order of increasing concentration of glutathione, the sequence is: dromedary, man, cattle, sheep, rat, guinea pig, rabbit (Lankisch *et al.,* 1973). GSH not only differs in its concentration among the different species but also among breeds, gender, organs, tissues, cells and cell compartments as well as different ages.

In this study our results suggested that there is a relationship between RBCs GSH with breed, gender and age. The RBCs GSH concentrations were significantly differ among the different studded camels breeds (Majaheem, Sofor, Shaele and Wodoh) of the Same sex at all studded ages (table 1,2&3), although Majaheem camels have the highest concentrations in both males and females while Shaele camels have the lowest concentration, this variation may be due to the genetic difference between the breeds. A similar results were obtained in deferent Indian buffalo breeds by Das and Majumder (2008). Majaheem camels are characterized by high growth rate and milk production that's consider a stressful factors so it has a well developed antioxidant system as adaptive mechanism. RBCs GSH concentration not significantly differ between Sofor and Wodoh females under five years (table 1) as well as Sofor with Shaele and Sofor with Wodoh males over 5 and 10 years respectively, the studded camels of these groups may be derived through cross breeding. This is the first study concerning the camel breed and RBCs GSH concentration so it needs more investigations.

Our result indicated that RBCs GSH concentrations were high in males when compared with females of all the studded species of the corresponding ages, this work was performed in December where the sexual desire of mature and activity of immature males are increased leads to increase of free radicals and as an adaptive mechanism the RBCs GSH levels increased.

Concerning the camel age the adult mature camels (4-10 years) (dromedary reproduction period)

either males or females and in all breeds had the highest RBCs GSH concentration when compared with the immature (1-4 year) or Aged camels (over 10 years) (table 4&5). Males of this group were sexually active so it consumes a lot of energy that produced from mitochondrial respirations with production of a lot of reactive oxygen species (ROS) with increasing of GSH and other antioxidant as an adaptive mechanism. All females of this group (4-10 years) were pregnant so they suffer from an excess of stress lead to increase of ROS production that need an increase of activity of antioxidant system especially RBCs GSH as a defense mechanism against ROS in pregnant women (Wisdom *et al.,* 1991; Casanueuva and Viteri, 2003) ewes (Mine *et al.,* 2009). The reports on GSH concentrations during pregnancy are controversial: some investigators showed an increase (Németh *et al.,* 2001), whereas others reported a decrease (Arikan *et al.,* 2001) or no significant change (Naziroglu *et al.,* 2004). Németh *et al*. (2001) suggested that the increased GSH concentration in pregnant women was considered as an adaptation to maintain the adequate redox milieu of the red blood cells. RBCs GSH concentration decreased with the increase of camel's age (table 4&5) we can reveal this to the efficiency of GSH synthesizing enzymes as glutamate-cysteine ligase (GCL),and glutathione synthetase. The *Km* of glutamate-cysteine ligase (GCL), the rate-limiting enzyme in *de novo* GSH biosynthesis, significantly increases during aging, which would adversely affect the ability for rapid GSH biosynthesis, especially under stressful conditions (Igor and Rajindar, 2008). Human RBCs GSH content was decreased in aged subject due to the decrease in GCL and GS activities (Honglei *et al.,* 2005). Typical parameters of aging process in rat are mainly the low levels of reduced GSH, total GSH and GSH Redox Index , probably contribute to decreases in the activity of the biosynthetic processes (i.e., NADP+(H) and GSH synthesis) and in the antioxidant capacity of the GSH system (Iantomasi *et al.,* 1993). The lowest content of nonprotein SH groups was observed in the young ewes. However, after a peak in the 31-50 months, it decreased dependently in older age ewes (Jamileh and Ali, 2010). Mice showed an age-dependent decrease in the GSH level in the brain and the liver (Jiankang and Akitane, 1993).

Conclusion:

In conclusion, the RBCs GSH concentrations were varied with in the Arabian camel breed, sex and age.

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