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DETERMINATION OF GLYCATED HAEMOGLOBIN (HBG) AND ITS CORRELATION WITH FASTING PLASMA GLUCOSE IN THE CAMEL

Homayon Reza Shahbazkia[1], Amin Bigham Sadegh[2], Mohammad Shadkhast[4], Afshin Jafari Dehkordi[3] and Mohammad Hossein Mahdavi[1]

[1]Department of Biochemistry, [2]Department of Veterinary Surgery and Radiology, [3]Department of Large Animal Medicine, [4]Department of Anatomy and Histology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord 115, Iran

ABSTRACT

Glycated haemoglobin (HbG) concentration is a retrospective measure of mean blood glucose level and is not affected by recent stresses, food ingestion or exercise. HbG has been determined in various wild and domestic animals such as kestrels, markhor, mouflon, aoudad, deer, goat, sheep, dog, camel and horse. But there is no information about HbG in Iranian cross-bred camels. On the other hand, relation of HbG to blood glucose and its value in metabolic profiling has not been clarified in the camel. The purpose of this study were to determine normal value of glycated haemoglobin in adult Iranian cross-bred camels and to investigate its relation to fasting plasma glucose. Blood samples were collected from jugular veins of 45 clinically healthy adult camels (20 males and 25 females). After separation and washing of red blood cells, hemolysate was prepared and subjected to weak cation exchange chromatography for determination of HbG. Glucose was measured in plasma samples. Plasma glucose and HbG in the camels were 109.7 ± 13.24 mg/dl and 4.12 ± 0.96% of total haemoglobin. It was shown that HbG% and plasma glucose correlated together ($r = 0.78$, $p<0.05$).

Key words: Camel, cation exchange chromatography, glycated haemoglobin (HbG)

When assessing nutritional, metabolic, physiological and pathological conditions, blood metabolic profile like blood glucose, insulin concentration and glucose tolerance tests are assessed. However, these are directly attributed to animals, condition and may be affected by stress, illness, pain and sexual cycle (Latimer *et al,* 2003). These reduce the value of such tests as an index of carbohydrate metabolism. Glycated haemoglobin (HbG) has been used in humans as a stable indicator of glucose status of healthy and diabetic patients (Gabbay *et al,* 1977; Jovanovic and Peterson 1981). Blood glucose can readily diffuse into erythrocytes (Latimer *et al,* 2003) and glucose in erythrocytes can react non-enzymatically with amino groups of haemoglobin (haemoglobin N-terminal or lysine side chain amino groups) to form HbGs. Formation of HbGs is essentially irreversible, and percentage of HbGs depends on the life span of erythrocytes and glucose concentration over the previous several weeks and is not affected by transient factors (Burtis and Ashwood, 1999). Hence, HbG concentration is a measurement of the mean blood glucose level over the previous several weeks and is not affected by recent stress, drug use or physiological conditions and therefore could be a better indicator for glucose status in comparison with other tests. HbG has been determined in various wild and domestic animals such as markhor (*Capra falconeri*), mouflon (*Ovis musimon*), aoudad (*Ammotragus lervia*; Richter, 1986), deer (*Odocoileus virginianus*; Jenks *et al,* 1991), kestrels (Ardia, 2006), goat, sheep (Alayash *et al,* 1988; Shahbazkia and Nazifi, 2008b), dog (Loste and Marca, 2001; Elliott *et al*, 1997), camel (Alayash and Wilson, 1987; Al-Ali *et al*, 1990), horse (Shahbazkia and Nazifi, 2005) and ostrich (Shahbazkia and Nazifi, 2008a). Although HbG has been determined in the camel (Al-Ali *et al*, 1990; Alayash and Wilson, 1987), there is no information about HbG level in Iranian cross-bred camels. On the other hand, correlation of HbG with blood glucose and its value in clinical biochemistry has not been studied in the camel. The purpose of this study was to determine HbG level and its correlation with fasting blood glucose concentration in the camel.

SEND REPRINT REQUEST TO HOMAYON REZA SHAHBAZKIA email: shahbazkia@yahoo.com

Materials and Methods

Forty five clinically healthy Iranian cross-bred camels (20 males and 25 non-pregnant females; after 10-12 hours of fasting) were sampled to identify plasma glucose and HbG. Blood samples were taken from the jugular vein and collected into vacuum containers and were immediately placed in melting ice and centrifuged at 2,000×g for 15 min. Plasma were collected and stored at -20°C until measurement of glucose. Erythrocytes were incubated with saline equal to the volume of the removed plasma for 30 min at 37°C to remove labile glucose adducts (pre-glycosylated haemoglobins) followed by centrifugation at 2,000×g for 10 min. Two volumes of cold water was added and mixed well to lyse red blood cells. To remove cell debris, the mixture was centrifuged for 15 min at 2,000×g. Haemolysate was removed and subjected to the next steps (Peterson *et al*, 1998). Glucose was measured using glucose oxidase and peroxidase. Neither haemolysis nor lipemia was detected in any of the plasma samples used for glucose measurement. For determination of HbG, a volume of prepared haemolysate was mixed with an equal volume of phosphate buffer (20 mM, pH 6.1), and 100 µl of this sample was loaded onto a chromatography column (1×5 cm) packed with cation exchanger resin (CM-Sephadex). Mobile phase was a linear gradient of 10 to 90 mM phosphate. The haemoglobin concentration in chromatography fractions and prepared haemolysates was determined using light absorbance at the wavelength of 415 nm. For *in vitro* glycosylation of haemoglobin, a volume of haemolysate was incubated with 400 mg/dl final concentration of D-glucose for 48 hours at room temperature. The HbG in glycosylated samples was determined by subjecting them to chromatography by the same method.

Results

Two major peaks were observed in the chromatogram of each haemolysate sample. The HbGs have smaller positive charge, so they should be eluted faster. To determine if the first peak was related to HbGs, *in vitro* glycosylated haemolysates, haemolysates incubated with glucose for 48 hours, were subjected to chromatography in the same conditions. It was shown that the first peak was spiked. Means within day and day-to-day coefficients of variation were 3.1 and 3.8%, respectively. We concluded that weak cation exchange chromatography with linear gradient of ionic strength was able to determine HbGs in sheep blood haemolysate. The normal values of HbGs in this study were 4.12 ± 0.96%. There was no significant difference between the HbG in males and females ($P>0.05$). Fasting plasma glucose concentrations were 109.7 ± 13.24 mg/dl in the studied camels. Our results showed that HbG percentage had positive correlation with plasma glucose ($r = 0.78$, $p<0.05$). The results of the regression analysis between fasting plasma glucose as dependent variable and HbG percent as a predictor are summarised in Table 2. R squared of the regression analysis was 0.95 and the standard error of estimate was 4.65. We found 4 camels with HbG percentage of 7.1%, 6.8%, 6.6% and 6.1% which exceeded the upper limit of the normal value obtained in this study. Fasting plasma glucose in these cases were 167, 153, 161, 138 mg/dl, respectively. Two times repeating the tests with 2 weeks interval showed the persistent high fasting plasma glucose and HbG percent in these camels indicating the diabetic status and value of HbG to diagnose diabetic camels.

Discussion

HbG was measured by weak cation exchange chromatography that we had introduced previously (Shahbazkia and Nazifi, 2005; Shahbazkia and Nazifi, 2008a,b). To validate our method, we measured the HbG in 10 normal humans using the same method and the results were within the normal range of human HbG (Burtis and Ashwood, 1999; Table 1). Means within day and day-to-day coefficients of variation showed that our method was reliable and reproducible. HbG percent in the studied camels was near to that of the human controls in this study (Table 1). In comparison with other studies, which determined HbG in human and other species, our data showed that the mean HbG in Iranian cross-bred (4.12%) was near to that of human (3–6%; Burtis and Ashwood, 1999), camel (4.39%; Al-Ali *et al*,

Table 1. Fasting plasma glucose and glycated haemoglobin in Iranian cross-bred camels and human controls.

Variable	Value	Unit
Plasma Glucose	109.7 ± 13.24	mg/dl
Glycated haemoglobin (HbG)	4.12 ± 0.96%	% of total Hb
Glycated haemoglobin in 10 human controls	4.92 ± 0.41	% of total Hb

Table 2. The results of the regression analysis between fasting plasma glucose (dependent variable) and HbG percent (predictor).

		Standard error	P value
Constant value	36.23	4.46	<0.01
Coefficient of the predictor	17.72	0.84	<0.01

1990), lower than camel (5.5%; Alayash and Wilson, 1987), and higher than goat (3.96%), sheep (3.24%; Alayash *et al*, 1988, 2.58%; Shahbazkia and Nazifi, 2008b), dog (3.3%; Elliott *et al*, 1997, 1.4%; Loste and Marca, 2001), horse (3.2%; Shahbazkia and Nazifi, 2005), ostrich (1.2%; Shahbazkia and Nazifi, 2008a) and kestrels (1.25%; Ardia, 2006). Theoretically, HbG percent is dependent to blood glucose concentration over the previous weeks, erythrocyte life span (Burtis and Ashwood, 1999), permeability of erythrocyte to blood glucose (Higgins *et al*, 1982) and potential of haemoglobin to condense with glucose. Therefore these between species variation in HbG can be explained by the variation of the mentioned factors among the studied species. Variation of the method by which HbG concentration was determined should also be considered as a factor making biases in between and inter- species HbG determination.

Glycated haemoglobin HbG concentration is a retrospective measure of mean blood glucose level and is not affected by recent stresses, food ingestion or exercise and its correlation with fasting blood glucose makes it more interesting to be considered as an indicator of blood glucose status. Therefore, significant strong correlation of HbG with fasting plasma glucose shows that the HbG can be considered as a good indicator of blood glucose status in the camel. We developed an estimating equation which predict mean fasting blood glucose from HbG percent; $FBG=17.72\times HbG + 36.23 \pm 4.46$.

In conclusion, we introduce HbG percent as a potentially good indicator of blood glucose status in camels. Considering 4 camels with persistent high fasting plasma glucose and HbG percent, we concluded that HbG percent could be a good biochemical test for screening the diabetes in camels.

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