Haemoglobin types and blood concentrations of haemoglobin, copper, ceruloplasmin and iron in adult Caspian miniature horses

S. NAZIFI and S. RATEGH

SUMMARY

The relationship between haemoglobin types of adult Caspian miniature horses and the blood concentrations of haemoglobin, copper, ceruloplasmin and iron was studied. Blood samples of 50 clinically healthy Caspian miniature horses with various ages (1.5-3, 3-5 and > 5 years) were taken from the jugular vein. By electrophoresis, two haemoglobin types were evidenced: the predominant type HbA and HbB, which respectively represented 66.58% and 33.42% of the total haemoglobin concentrations. Proportions of haemoglobin types, haemoglobin concentrations, and serum iron, copper and ceruloplasmin concentrations were independent of age or sex, and no significant correlation among these biochemical markers of iron metabolism could be obtained. The interest of this study was to identify 2 haemoglobin types in adult miniature Caspian horses and to dispose of normal serum iron, copper and ceruloplasmin concentrations in this horse breed. The identification of regulatory mechanisms of iron metabolism will require further investigations.

Keywords : ceruloplasmin - copper - haemoglobin type - iron - Caspian miniature horse.

Introduction

Perhaps, the most exciting equine discovery of the 20th century was that the Caspian is not a pony, but an ancient breed of miniature horse, previously believed to have been extinct for over a thousand years. These animals native of the areas around the Elborz Mountains and Caspian Sea in Iran, are extremely rare and were rescued from extinction in 1965 by an American L.FIROUSE [11]. The current theory is that Caspian horse is the ancient miniature horse of Mesopotamia, which was considered as extinct until the 7th century Anno Domini (AD) [7]. But, this tiny horse was probably one direct ancestor of the oriental breeds and subsequently of all modern breeds of hot-blooded horses [5, 8, 11]. For confirming this new theory, the study of haemoglobin types in this species would be useful. In this respect, MCCLURE and PARISH [17] reported that electrophoretic markers (such as haemoglobin) are useful genetic markers for identification and parentage studies. Haemoglobin types have been studied in Indian horses, quarter horses, Caspian miniature horses and Turkoman horses [9, 18, 20, 27]. The majority of horses have two types of haemoglobin, but certain breeds may present only one component [13]. Two haemoglobin types (A1 with high mobility and A2 with low mobility) were evidenced in Indian horses [27], in Turkoman horses [20], in Arab horses [24] and in quarter horses [9] in which the high mobility type (A1) was predominant.

Copper is essential for haemoglobin formation and iron transport. Copper is the rate-limiting element in the synthesis of ceruloplasmin, a glycoprotein that is synthesized in the liver. Ceruloplasmin acts as a ferroxidase, by oxidizing Fe^{+2} into Fe^{+3}, and allowing Fe^{+3} to bind with the iron transport protein, transferrin. Ceruloplasmin concentrations have been considered as reliable indicators to copper status [13]. KIN-CAID et al. [16] reported that serum ceruloplasmin was closely correlated to both serum and plasma copper concentrations. By contrast, FAYE et al. [10] reported that in the camel, the correlation between ceruloplasmin and copper plasma concentrations was low. The relationship between the types of haemoglobin and the concentration of minerals (iron and copper) in the blood of different breeds of sheep from different geographical locations was reviewed by AGAR et al. [1]. NAZIFI et al. [20] reported that there was no significant correlation between the concentration of haemoglobin, copper, iron, ceruloplasmin and haemoglobin types in adult Turkoman horses. Studies on the relationship between copper, ceruloplasmin, iron, haemoglobin and haemoglobin types will increase our understanding on the metabolism of blood components in various species. The potential establishment of such correlations in an ancestral equine breed, the miniature Caspian horse, would be out of interest. The aim of the present study is to determine whether there is any correlation between these parameters in this particular horse specie.
Materials and methods

Blood samples were collected from 50 adult Caspian miniature horses according to their age (1.5-3, 3-5 and >5 years) and sex. The horses had been reared in the province of Golestan, northeastern Iran. All the animals were clinically healthy and free from internal and external parasites. Each horse has a separate file including all necessary records, and the age of the horses was determined by referring to these records. For the haemoglobin determination and electrophoresis, blood samples were collected by jugular venipuncture into vacuum containers containing EDTA. For the analysis of serum copper, ceruloplasmin and iron, blood samples were collected into plain vacutainers and the serum was separated following centrifugation for 15 min at 750xg at room temperature. Any haemolysed samples were discarded. Serum samples were stored at -20°C until analysed.

The haemoglobin concentration was determined by the Cyanmethaemoglobin method [12]. For the evaluation of the haemoglobin types, haemolysates were prepared by washing packed red blood cells three times with isotonic saline and then haemolysing with an equal volume of distilled water. Haemoglobin types were determined by electrophoresis on cellulose acetate, using Tris - EDTA borate buffer (pH=8.5). Voltage was maintained at 350V and current increased from 4 to 10 mA over 45 min. The samples were analysed for copper and iron by atomic absorption spectrophotometry (Shimadzu AA-670, Kyoto, Japan). Ceruloplasmin activity was measured according to its phenylenediamine oxidase activity [28].

Means, standard errors, correlation coefficients and significances were determined using the general linear model (GLM) procedure of the SAS program [25]. All values were expressed as mean and standard error (SE), and results were considered as significant when P values were less than 0.05.

Results and Discussion

Two haemoglobin types were evidenced by electrophoresis (Figure 1, Table I): HbA and HbB. HbA was the major predominant type identified in adult Caspian miniature horses and represented 66.58% of the total haemoglobin concentration, whereas HbB represented 33.42%. No relation was found between haemoglobin types and age or sex. MOHRI et al. [18] reported two haemoglobin types in Caspian miniature horses, A and B. Two haemoglobin types (A1 with high mobility and A2 with low mobility) were also evidenced in Indian horses [27], in Turkoman horses [20] and in Arab horses [24] in which the high mobility type (A1) was predominant. JAIN [13] reported that the majority of horses have two types of haemoglobin, but that certain breeds may have only one component. EZCURRA and MITAT [9] studied the haemoglobin of quarter horses by starch gel electrophoresis and also reported two types, A1 and A2, with A1 predominant. NISHIMURA et al. [21] reported the occurrence of three haemoglobin phenotypes for southeast Asian ponies. Horses generally have two types of haemoglobin, A1 and A2, in the ratio of 2.5:1 [12, 13]. The ratio in Caspian miniature horses for HbA and HbB was 1.99:1, and therefore lower than the ratio reported for other breeds [12, 13]. Consequently, there is no HbB type in modern horses, suggesting that this haemoglobin variant has been replaced by a different haemoglobin in amino-acid composition, HbA2, after gene mutation during evolution [12].

The means ± SE of copper, ceruloplasmin, iron, haemoglobin concentrations obtained in adult Caspian miniature horses are presented in Table I. Haemoglobin concentrations in Caspian miniature horses were 113.22 ± 2.12 g/l. Blood haemoglobin concentrations observed in adult Caspian miniature horses were similar to values reported earlier [4, 12]. No significant variations according to age (Table I) or to sex were noticed. On the contrary, ALLEN and ARCHER
[2] observed variations of haemoglobin concentrations, red blood cell (RBC) counts and packed cell volume (PCV) in Thoroughbred horses according to age; haemoglobin concentrations and PCV were lowest in 1-9 month old foals, and then gradually increased until 4 years of age, whereas RBC counts fluctuated in an opposite way. Consequently, red blood cells grew and became saturated in haemoglobin with age, leading to reduction of their number. But, such an age-related decline in red cell numbers was not seen in training older horses [3]. JAIN [12] reported that females of both Thoroughbred and quarter horses presented RBC counts, Hb concentration and PCV values weakly greater than males.

Iron and copper serum concentrations were 14.45 ± 0.11 and 13.04 ± 0.13 µmol/l respectively. The mean concentration of serum copper of adult Caspian miniature horses was similar to the values reported for quarter and Turkoman horses [14, 20]. Mean serum iron concentration recorded in this study was similar to values reported by others in horses [6, 23]. Although copper concentrations seemed to weakly decreased with age (Table I), age and sex have exerted no significant effect on serum mineral concentrations. These results are in good agreement with previous studies which reported no influence of age on sideremia in calves [15] or in horses [26]. A weak but not significant decrease of serum ceruloplasmin concentrations was noticed in older horses (> 5 years: 301.4 ± 2.1 µmol/l) whereas ceruloplasmin concentrations in 1 - 5 year old horses reached 304 to 306 µmol/l (Table I). No difference was observed between males and females. The serum ceruloplasmin concentrations of Caspian miniature horses were comparable to those of Turkoman horses [20], Arabian camels [10] and Iranian camels [19], but were higher than values reported for cattle [16]. Our study showed that, age had no significant effect on this parameter in adult horses. By contrast, serum ceruloplasmin concentrations were minimum in one week old foals, then rapidly increased during the first month of life and remained constant in one year old horses [22].

In adult Caspian miniature horses, no significant correlation was obtained among all the studied biochemical markers, particularly haemoglobin types did not correlate with serum concentrations of minerals (iron and copper) and ceruloplasmin. These results confirmed previous studies conducted on Iranian camels [19] and on Turkoman horses [20] which also failed to evidence significant correlations between haemoglobin concentrations or haemoglobin types (A1 and A2) and copper/iron or ceruloplasmin concentrations. FAYE et al. [10] observed a low correlation between plasma ceruloplasmin and copper concentrations in camels, whereas KINCAID et al. [16] reported a significant positive correlation between serum copper and ceruloplasmin concentrations in cattle (r² = 0.48). Perhaps, the sizes of horse population samples in these different studies were too low for evidencing direct relations between biochemical markers of the iron metabolism. No other explanation for the lack of proportionality among these markers is actually available and further investigations are needed to clarify this point.

References