Postnatal Assessment of Serum Amyloid A Concentrations and Biochemical Profiles In Lactating Female Donkeys and Newborn Donkey Foals

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Abstract

Objective:Proper knowledge of biochemical parameters and inflammatory markers like serum amyloid A (SAA) is crucial in monitoring the first postpartum period in equids. Materials and Methods:Since no information is available on SAA for donkeys at this stage, 100 animals including lactating female donkeys (n.20) and newborn foals (n.20) within 48h from foaling, and lactating female donkeys (n.20) and foals (n.40) after 30 days from parturition were enrolled in the study to assess routine biochemical profile including SAA. Results:Lactating female donkeys showed higher alkaline phosphatase and lower bilirubins and cholesterol at 30 days of lactation compared to postpartum. Neonatal donkey foals showed significantly higher concentrations of sodium, alkaline phosphatase, lactic dehydrogenase, blood urea nitrogen, creatinine, and albumin within 48h of age. In contrast, higher values of phosphate and triglycerides were observed in older foals of 30 days of age. Significant higher SAA concentrations were recorded during the peripartum period in both lactating female donkeys (27.69±1.67 μg/ml) and newborn donkey foals (39.62±18.58 μg/ml) compared to SAA values recorded in lactating female donkeys(13.59±2.76 μg/ml) and in donkey foals (15.87±19.42 μg/ml) at 30 days after parturition. Conclusion:Lactating female donkeys and foals were tested and assessed for SAA before and after parturition and one month postpartum. The assessment results are an important basis for monitoring the health of lactating female donkeys and foals at this stage, and now they are also facing new challenges, such as the peak lactation period of lactating female donkeys and the adaptation of the foal to the extra-uterine environment.

Introduction

Changes in animal metabolism are affected by many specific conditions, such as pregnancy, parturition, and lactation. A large number of data show that we have deeply studied the blood parameters and biochemical indicators of mares during the perinatal period(Bazzano et al., 2014; Mariella et al., 2014; Bonelli et al., 2016). Still, there are few reports on the research on lactating female donkeys. However, there are differences in the biochemical parameters of healthy pregnant subjects between different species. In order to clarify the reference range of biochemical indicators of lactating female donkeys at this stage and make up for the shortcomings of research in this field, this study was done(Sgorbini et al., 2013;). At the same time, when evaluating various indicators in foals and donkeys, clinicians need to be aware that the specific reference ranges for reference vary depending on the age of the animal(Veronesi et al., 2014).

Beginning a few years ago, specific acute phase protein tests have been added to routine hematology and biochemical evaluations, allowing clinicians to more quickly identify and differentiate acute infections, inflammation, and other more clinical symptoms through the test results(Kay et al., 2019). Serum amyloid A (SAA), an acute-phase protein of the apolipoprotein family (APP), is mainly produced by the liver and increases rapidly when symptoms of inflammation occur. SAA is the only major positive APP in horses because its concentration is low or clinically undetectable in normal animals, but rapidly increases from 10 to 1000 fold after the onset of the acute phase response(Long & Nolen-Walston, 2020; Nolen-Walston, 2015).

At the same time, because of its very short half-life (30-120 minutes), the concentration of SAA will increase after 6 hours of stimulation and decrease within 12 hours of the end of the disease(Long & Nolen-Walston, 2020). Prior to this, other research teams have statistically analyzed the changes in SAA concentrations in horses under different conditions, such as: respiratory diseases, colic, orthopedic diseases and after surgery, etc(Witkowska-Piłaszewicz et al., 2019b). A team of researchers recently reported an analysis of the impact of reproductive diseases on SAA in mares(Coutinho da Silva et al., 2013; Krakowski et al., 2020;). There are also related reports on the SAA indicators of healthy horses under different types of exercise conditions or under special physiological conditions (brooding period and perinatal period)(Piccione et al., 2016; Witkowska-Piłaszewicz et al., 2019b). However, there are no statistics about donkeys in this regard.

At present, the donkey breeding industry is getting bigger and bigger in the world. The correct use of donkey medicine within a specific reference range is gaining more and more attention. But there is no relevant information on the SAA indicators of healthy donkeys. Due to the importance of SAA indicators in the early diagnosis of equine inflammatory diseases, and the huge economic losses caused by the disease of lactating female donkeys and foals, research on the early diagnosis and treatment of donkey inflammation is very urgent(Jerele et al., 2020; Kay et al., 2019; McLean et al., 2016).

Based on the current knowledge, this study was mainly to analyze the main biochemical parameters and SAA levels within one month after parturition in lactating female donkeys and newborn foals in Dezhou, China.

Materials and Methods

Animals

All animal housing, care and experimental procedures herein described were in accordance with the standards recommended by the EU Directive 2010/63/EU for experiments on animals. The research protocol was approved by Internal Animal Welfare Committee (approval number 7/2021).

A total of 100 Chinese Dezhou donkeys raised on the same farm in Dezhou, China participated in this experimental study with informed consent from ranchers. All subjects participating in the study have passed the clinical examination, and all subjects are guaranteed to be in a healthy state. The donkeys are divided into four groups according to the sampling time: group A consisted of n.20 lactating female donkeys within 48 hours after parturition, group B consisted of n.20 lactating female donkeys lactating for 30 days, group C consisted of n.20 lactating female donkeys Heads consisted of (12 males, 8 females) neonatal foals (within 48 hours after birth), and group D included n.40 (22 males, 18 females) foals (within 1 month) (Table 1). Lactating female donkeys in groups A (mean age 7.5±2 years, mean BCS 2.8±0.2) and B (mean age 7.7±2.5 years, mean BCS 2.5±0.2) were fed 6 kg of hay per day and 0.5 kg/day of concentrate (Evans & Crane, 2018). During the experiment, female donkeys were fed the foals of groups C and D for 24 hours. For the first week of life, foals are housed individually in incubators with soft straw beds. After that, like other foals, they are transferred to the public paddock for unified feeding. At the same time, all subjects participating in the study were grazed for 8 hours a day and provided with sufficient water.

During October 2020, blood samples were collected during routine veterinary procedures at the donkey farm in the morning prior to feeding (8am-9am). A single blood sample was drawn from the jugular vein of each animal participating in the study into a 10 mL tube containing blood anticoagulant.

Laboratory analysis

After blood samples were collected, they were stored in a 0°C incubator and sent to the laboratory in time. In the laboratory, samples were centrifuged at 1,000 g (Universal 32, Hettich Zentrifugen, Germany) for 10 min, and serum samples obtained were divided into two 1.5 mL aliquots, stored at -20°C for unified analysis studies.

Various indicators in the serum of the samples were detected by an automatic clinical chemistry analyzer BT 3500 VET plus: Potassium (K), Sodium (Na), Chloride (Cl), Calcium (Ca), Phosphorus (P), Calci-

um/Phosphorus ratio (Ca:P) blood urea nitrogen (BUN), γ-glutamiltrasferase (GGT), glucose (Glu), creatinine (Cre), glutamic oxaloacetic trans-aminase (GOT), serum glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), cholesterol (Chol), triglyceride (Trig), creatine kinase (CK), total bilirubin (tBil), direct bilirubin (dBil), indirect bilirubin (iBil), total protein (TP), albumin (Alb), globulins (G) Albumin/Globulin ratio (Alb:G).

Serum Amyloid A (SAA) has been detected in serum samples by a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using the multispecies Tridelta PhaseTM range SAA kit. In other words, SAA-specific monoclonal antibodies are coated with the wells of the microtiter strip provided with the kit. All SAAs will be discovered and captured during this time. Wash and add the chromogenic substrate 3,3',5,5'-tetramethylbenzidine. A large amount of blue product is produced, which is positively correlated with the amount of SAA in donkey serum samples. The color reaction needs to be terminated by a stop solution and the color intensity of the product is measured with a microtiter plate at a light wave of 450 nm. Concentrations of test samples were obtained from a calibrated semi-log standard curve and expressed as mean concentration (μg/ml) ± SE. As indicated in the datasheet, intra-assay (within) precision/reproducibility and between-batch precision/reproducibility have been tested to validate the test.

Statistical analysis

Data were analyzed using statistical software Prism 8 (Graphpad Software Ltd, USA).

Student T-tests were performed to highlight significant differences in studied blood parameters between Group A and B, and between Group C and D, respectively. Values of $P < 0.05$ were considered statistically significant.

Statistical analysis was performed with a one-way analysis of variance (ANOVA), followed by the Bonferroni test using Sigma-stat 3.1 software (SPSS, Chicago, IL, USA). Significantly different values ($P<0.05$) were indicated in bold letters.

Results

All lactating female donkeys included in the study delivered at term (mean gestation length 355±19 days), by spontaneous eutocic parturition, healthy viable foals.

After summarizing and analyzing all the data, it was found that the SAA concentration value of group A and group C was significantly higher than that of group B and group D after 48 hours of delivery. And other biochemical parameters were found to be different among the groups. Compared with group A, group B had higher ALP value but lower values of Chol, tBil, dBil and iBil. Compared with group D, group C had higher Na, BUN, Crea, ALP, LDH, Alb and Ca/P values in donkey serum but lower P and TG values.

Statistical results of donkey serum biochemical parameters in groups A and B are shown in Table 2, and groups C and D are shown in Table 3.

Discussion

Before this, some research teams have studied and reported the blood characteristics of donkeys during the perinatal period (Veronesi et al., 2014). However, based on the available data, we are the first team to use SAA as a routine biochemical indicator to evaluate the health of lactating female donkeys and newborn foals (Sgorbini et al., 2013).

Comparing the statistical results with those reported by other research groups, we found almost no significant changes in the serum biochemical parameters of female donkeys during lactation (Milonis & Polidori, 2021). The existing literature indicates that the serum cholesterol of lactating female donkeys or mares decreases after 30 days of lactation, which is very similar to the statistical results observed in our study. And we know that the fat content of donkey milk is lower than that of other species of milk, however, the cholesterol content of donkey milk in this study ranged from 0.41g/100g to 0.97g/100g, which is higher than that of ordinary milk (Evans & Crane, 2018). Compared with the research results of other teams, the serum Alp

value of the donkeys participating in this experiment was higher, and the field increased during lactation, which was significant. The authors speculate that this phenomenon occurs, similar to the reason for the increase in serum Alp value during lactation in other mammals, which may be due to the effect of mammary ALP on the activity of Alp in serum to a certain extent. At the same time, we found that the mean bilirubin was higher than the normal range given in the reference, but after 30 days of lactation, the value returned to the normal range (Bazzano et al., 2014; Mariella et al., 2014). Although we did not collect blood samples from late pregnancy for testing, this phenomenon is similar to that of perinatal mares, and the reason for the high bilirubin value should be due to cholestasis.

As reported by other research groups, the newborn foals in the experiment experienced significant changes within one month after birth, with unstable blood biochemical indicators, such as a decrease in BUN and creatinine (Sgorbini et al., 2013). Foal ALP values also continued to decline at one month of age, although we mentioned earlier that the ALP average was higher than the reference range, a phenomenon similar to that of the Amiata and Martina Franca foals (Veronesi et al., 2014). Compared with the Martina Franca foal, there are also differences in blood parameters, such as a significantly lower LDH value and a higher TG value, which also shows that the biochemical parameters of different types of foals cannot be referenced. At the same time, the electrolyte data in the samples showed that the Na content and albumin content were higher in the newborn foal compared with the one-month-old foal.

During this study period, we captured significant changes in SAA values of lactating female donkeys and foals. According to the available data, the stable range of SAA values in healthy horses in equines is 0-20 ug/mL, and there is no gender difference in this value range. And the average SAA of one-week-old foals was 27.1ug/mL. According to our statistical results, the average SAA value of 2-days-old healthy foals was 39.62±18.58 ug/mL. Therefore, it can be seen that compared with the neonatal horses reported in the reference, healthy neonatal donkey foals have higher concentrations of SAA. The authors speculate that the physiological differences are caused by different experimental animals or different time of sample collection. Statistical analysis showed that the concentration of SAA in lactating female donkeys changed significantly during the perinatal period. The SAA value was 27.69±1.67 ug/mL within 48 hours after delivery, and decreased to 13.59±2.76 ug/mL after 1 month of lactation. Compared with other teams' studies on pregnant mares, SAA concentrations remained stable for 4 months before parturition, but from 7 days before parturition to about 30 days after parturition, due to fetal displacement during this period, some tissue damage was caused. As a result, the SAA concentration increased significantly. SAA concentrations increased significantly at 12 hours (0.7-305 mg/L) and 36 hours (0-1615 mg/L) after delivery, and returned to the normal range and remained stable after 60 hours (Witkowska-Piłaszewicz et al., 2019a). Similar studies by other researchers have shown that mares who gestation in a healthy state and give birth to healthy foals have stable and unchanged SAA concentrations after parturition.

Although different research methods can have different effects on SAA values, in mammals, SAA concentrations increase significantly during parturition because this APP is involved in the process of partition (Bordonaro et al., 2013). According to the research results made by Gan and his team, SAA participates in the labor process by releasing and stimulating the expression of inflammatory factors related to labor through the placenta, thereby increasing the production of pro-inflammatory cytokines to participate in the labor process, which is independent of external environmental infection (Gan et al., 2020). However, from our own research data, healthy newborn foals have higher SAA concentrations within 48 hours after parturition than lactating female donkeys, and we believe that the reason is due to the physiological response caused by the difference between the external environment and the intrauterine environment.

There are also some problems in this experimental study: the subjects participating in the experiment are all from the same farm. Although the sample size and statistical evaluation are consistent, there are still flaws in obtaining the SAA concentration range, which cannot be regarded as the standard range. In order to improve the shortcomings of this field, we believe that the sample size and donkey species should be expanded, and further experimental research should be conducted.

Conclusions

The physical health of female donkeys before and after parturition and lactation, as well as newborn healthy foals, can be assessed by early SAA testing, which is very useful for us to understand their physical condition. At present, we have very difficult challenges to monitor the health of new born foals and female donkeys after giving birth. It is difficult for large livestock to express early symptoms, and it is difficult for us to detect them in time due to the insignificant clinical symptoms, thus making the disease worse. For the clinical stage, we need an accurate reference range of SAA concentration values to detect and compare donkeys. Nevertheless, the information provided by this experiment can still provide preliminary help for the early diagnosis of lactating female donkeys and foals and save important economic losses to ranchers.

Conflict of interest

Authors declare no conflict of interest.

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