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Original Research

Comparison of Refractometric and Biuretic Methods for the Assay of Total Protein in Horse Serum and Plasma Under Various Storage Conditions

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ABSTRACT

This study aimed to investigate the differences in assessment of total proteins between the use of refractometric and biuretic methods in equine serum and plasma samples and to test the validity of the two methods at different storage conditions. Serum and plasma total proteins concentration from 60 nonhemolytic and nonlipemic equine blood samples was assessed using refractometric and biuretic methods. On serum and plasma samples albumin, triglycerides, cholesterol, glucose, total bilirubin, and fibrinogen values were also evaluated. Samples were analyzed immediately after centrifugation (T0), refrigerated at 4°C and analyzed after 24 hours (T1) and 48 hours (T2), stored under refrigeration at 4°C, and after 1 week (T3) of storage at −20°C. No statistically significant effect of method ($P > .05$) on serum and plasma total proteins levels was found. A significant effect of storage condition ($P < .01$) and sample type ($P < .05$) was revealed on total proteins values. A significant effect of storage condition ($P < .01$) was found on albumin, triglycerides, glucose, and total bilirubin values measured in both serum and plasma samples. Significant lower fibrinogen values were found at T0 with respect to T1, T2, and T3 and at T1 and T2 with respect to T3 ($P < .001$). The use of refractometer for total proteins measurement in equine serum or plasma samples without any visible abnormality was sufficiently reliable, providing an inexpensive and rapid alternative to biuret method. The present study suggests that storage conditions of serum/plasma samples are important preanalytical variables, which may considerably affect the determination of the most frequently texted parameters in the equine species.

1. Introduction

In veterinary medicine, the assessment of total protein concentration is one of the most frequent routine analyses performed to investigate many pathological conditions. Accurate measurement of clinical biochemistry parameters is an essential tool for the correct interpretation of clinical biochemistry abnormalities [1–4]. The routine measurement of plasma/serum total protein concentration is commonly performed by biuret method based on the formation of copper chelates by the enolized

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peptide bonds of proteins at alkaline pH [5]. The biuret reaction can be performed in liquid reagents or with "dry chemistry" reagents. The latter are the most frequently used in the analyzers of veterinary clinics. Another total proteins measurement often used in veterinary clinical pathology is based on refractometry. Refractometer, allows rapid and inexpensive determination of solute concentrations in various fluids by means of the measure of the angle of refraction between air and aqueous solution [6]. The angle of refraction produced by a serum or plasma sample is due to the combined concentration of all its solute, also called total solids. The predominant solute is a protein, but a nonprotein substance contributes to the angle of refraction; because of this reason, protein and total solids are not interchangeable terms [6]. Differences in total protein concentration can be observed with different types of refractometers and with interference from various solutes [7]. In domestic animals, differences between the results of refractometer and biuret techniques have been reported [5]. Controversy exists regarding the relative contributions of glucose, cholesterol, urea, sodium, and bilirubin levels to the nonprotein component of total solids and, consequently, the clinical significance of increases in these parameters with respect to the refractometric total protein measurement [8]. It has been proposed that the nonprotein solids, including glucose, cholesterol, and urea, lead to the increase of total proteins concentration as determined by the refractometer in avian [9].

Considering that in an equine clinical practice, there is the need to carry the samples from the stable to the laboratory, optimal management of the preanalytic phase is crucial [10]. Accordingly, the extraanalytical variables affecting the analytical process, including transport conditions, protract storage at high or low temperature, and improper handling (without frozen packs), have to be carefully considered and standardized during the processing of the sample [11,12]. It is highly recommended to perform tests from freshly drawn serum or plasma because the delay in the analytical process or the reuse of samples for missing results may cause false concentrations of the texted parameters [13–15].

It has been hypothesized that the refractometer could be a reliable and easy method for routine screening of serum/plasma total protein concentrations in equine; however, the storage of biologic samples could be a potential source of preanalytic errors and must be taken into account to avoid erroneous results.

Therefore, the present study aimed to investigate the differences in the assessment of total proteins between the use of refractometric and biuretic methods in equine serum and plasma samples, to test the validity of the two methods at different storage conditions and to evaluate whether the concentration of some nonprotein substances including albumin, triglycerides, cholesterol, glucose, total bilirubin, and fibrinogen resulted correlated with the values of total proteins measured by refractometer and biuret methods.

2. Materials and Methods

2.1. Animals

All treatments, housing, and animal care were carried out in accordance with the standards recommended by the European Directive 2010/63/EU for animal experiments.

Sixty clinically healthy and regularly trained Thoroughbred horses (35 geldings and 25 females) aging between 3 and 7 years and a mean body weight 395 $\pm$ 16 kg, were enrolled in this study after the informed consent had been provided by the owner.

The horses were considered healthy if they did not have a history of change in hemostatic mechanisms and if their physical examination as well as packed cell volume, white blood cells, and platelet count were within normal limits. No pharmacologic treatment was administered for 1 month before the study.

The horses enrolled in the study were trained 6 days a week with a rest day on Sundays. Training included walking (30 minutes), trotting (20 minutes), and galloping (5 minutes).

All animals were housed in individual boxes (3.50 $\times$ 3.50 m) under natural spring photoperiod (sunrise at 06:00 hours and sunset at 18:00 hours), at an indoor temperature of 18–21°C.

All animals were fed with standard rations, consisting of hay (first cut meadow hay, sun cured, late cut, average 8 kg/horse/day) and a mix of cereals (oats and barley, 50% each, about 3.5 kg/horse/day) provided three times a day (at 7:00, 12:00, and 19:00). Water was available ad libitum.

2.2. Blood Sampling and Laboratory Analysis

From each animal, blood samples were collected by jugular venipuncture into 10 mL vacuum tubes containing lithium heparin (Terumo Corporation, Tokyo, Japan), 3.6 mL vacutainer tubes (Terumo Corporation) containing 3.8% of sodium citrate with a blood to anticoagulant ratio of 9:1, and into 9 mL vacutainer tubes containing clot activators (Terumo Corporation).

After storage at room temperature for 20 minutes, the vacuum tubes containing lithium heparin and clot activators were centrifuged at 1308 g for 10 minutes using the Thermo Scientific CL10 centrifuge (Thermo Fisher Scientific Inc, Waltham, MA) to obtain plasma and serum samples, respectively.

Each sample was then divided in four aliquots. To be used for the analytical determinations, the obtained serum and plasma samples had to be neither hemolyzed nor lipemic. One aliquot was analyzed immediately after centrifugation (T0). The second and the third aliquots were refrigerated at 4°C and analyzed after 24 hours (T1) and 48 hours (T2), respectively. The last aliquot was stored at −20°C (frost-free freezer: daily temperature range, −18°C to −20°C) after centrifugation and analyzed after 1 week (T3). The serum and plasma total proteins concentration was measured using a refractometer (Clinical Refractometer T2-Ne; ATAGO CO, LTD, Tokyo, Japan; protein scale