Research, Society and Development, v. 9, n. 5, e18952619, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i5.2619

Validação de parâmetros do perfil renal e hepático de roedores *Rattus norvegicus* do Biotério de manutenção da Universidade de Franca

Validation of renal and hepatic profile parameters of rodents *Rattus norvegicus* kept in the maintenance vivarium of the University of Franca

Validación de los parámetros de perfil renal y hepático de roedores *Rattus norvegicus* mantenidos en el vivero de mantenimiento de la Universidad de Franca

Recebido: 21/01/2020 | Revisado: 10/02/2020 | Aceito: 19/03/2020 | Publicado: 26/03/2020

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Resumo

Devido ao alto uso de animais como modelos experimentais para ensaios farmacêuticos, principalmente ratos, é necessário padronizar valores de referência para avaliação do perfil hepática pelos analitos alanina aminotransferase (ALT) e aspartato aminotransferase (AST) e função renal, através dos analitos uréia e creatinina. Para tanto, foram coletadas amostras de 169 amostras de *Rattus norvegicus* da linhagem *Wistar*, do Biotério da Universidade de Franca (SP), realizados com metodologia analítica semi-automatizado. Para o cálculo do valor, foram realizados média e desvio padrão para padronização e criação de valores de referência, sendo utilizados -2DP para determinação do valor mínimo e + 2DP para o valor máximo. A enzima hepática ALT apresentou uma faixa de referência de 21 a 96 U / L, enquanto a AST de 44 a 186 U / L. Nos marcadores renais, a uréia foi de 20 a 73 mg / dL e a creatinina de 0,23 a 0,77 mg / dL. Dada a metodologia aplicada e os resultados obtidos, admite-se que é muito importante padronizar os valores de referência dos analitos em biotérios de animais devido a alterações geográficas, protocolos, dietas, entre outros, além disso, reduzindo o uso de animais em procedimentos experimentais, além de orientar pesquisadores sobre saúde animal e auxiliar a análise em vários projetos de pesquisa experimental.

Palavras-chave: bioquímica clínica; biomarcadores; biotérios; enzimas séricas; ratos.

Abstract

Due to the high use of animals as experimental models for pharmaceutical trials, especially rats, it is necessary to standardize reference values for the evaluation of liver perfil by the analytes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and renal function, through the analytes urea and creatinine. For this purpose, samples were collected

from 169 samples of *Rattus norvegicus* of Wistar strain, from the Vivarium of the University of Franca (SP), provided of semi-automated analytical methodology. For the calculation of the value, mean and standard deviation were performed for standardization and for the creation of reference values, therefore -2DP for minimum value determination and + 2DP for maximum value were used. The liver enzyme ALT presented a reference range from 21 to 96 U / L, while the AST from 44 to 186 U / L. In renal markers, urea was 20 to 73 mg / dL and creatinine 0.23 to 0.77 mg / dL. Given the applied methodology and the results obtained, it is admitted that it is very important to standardize the reference values of analytes in animal houses due to geographical changes, protocols, diets, among others; In addition, reducing the use of animals in experimental procedures in addition to guiding researchers on animal health and assisting analysis in various experimental research projects.

Keywords: clinical biochemistry; biomarker; vivarium; serum enzymes; rats.

Resumen

Debido al alto uso de animales como modelos experimentales para ensayos farmacéuticos, principalmente ratas, es necesario estandarizar los valores de referencia para la evaluación del perfil hepático por los analitos alanina aminotransferasa (ALT) y aspartato aminotransferasa (AST) y la función renal, a través de los analitos de urea y creatinina . Para ello, se recogieron muestras de 169 muestras de Rattus *norvegicus* de la espécie Wistar, del Vivarium de la Universidad de Franca (SP), realizadas con metodología analítica semiautomatizada. Para el cálculo del valor, se realizaron promedios y desviaciones estándar para la estandarización y la creación de valores de referencia, utilizando -2DP para determinar el valor mínimo y + 2DP para el valor máximo. La enzima hepática ALT mostró un rango de referencia de 21 a 96 U / L, mientras que la AST de 44 a 186 U / L. En los marcadores renales, la urea fue de 20 a 73 mg / dL y la creatinina de 0.23 a 0,77 mg / dL. Dada la metodología aplicada y los resultados obtenidos, se admite que es muy importante estandarizar los valores de referencia de analitos en viveros animales debido a cambios geográficos, protocolos, dietas, entre otros, además de reducir el uso de animales en procedimientos experimentales. , además de guiar a los investigadores en salud animal y ayudar al análisis en varios proyectos de investigación experimental.

Palabras clave: bioquímica clínica; biomarcadores; viveros; enzimas séricas; ratas.

1. Introduction

The use of experimental animals is very important for the worldwide development of science and, despite the alternative methods already established in the literature, rats are still considered reliable models for several pharmaceutical trials (Lima et al., 2014). The use of the animal model for experimentation was an alternative created to overcome the ethical and operational obstacles in the field of experimental pathology, allowing the search for knowledge of the etiology and the mechanisms involved in several pathologies, and later, allowing the results obtained to be extrapolated for application in humans (Fagundes and Taha, 2004; Almeida Junior, 2019).

In this context, it is extremely important that the breeding, maintenance and experimentation facilities have a standardization of the physiological profile of the biochemical values of their animal models, in order to guarantee the safety and health of the animals used to increase the reliability of the results of the animals, treated and controls, since these values can be changed due to the experimental conditions presented (Dantas et al., 2008, Castello Branco, 2011), interfering with therapeutic approaches (Almeida Jr, 2014).

In order to guarantee reliability in clinical research, it is of great importance to standardize the experimental model, and also of all the processes involved in the study until obtaining the results, where the error is present at any stage, can induce the variation of the final results (Dantas et al., 2008, Almeida Jr, 2014).

As far as laboratory analysis is concerned, there are three important steps to be observed, in which it is prone to errors: pre-analytical (reception, collection and transport of samples), analytical (exams) and post-analytical (review and release of the report) (Lima-Oliveira et al., 2009), being the pre-analytical with the highest number of errors (for 68.2% of the failures) (Plebani, 2009), directly involving manual work as an option for needle gauge and collection tubes (Vieira et al., 2011; Junior et al., 2020), in addition to storage and transportation of rates (Almeida Jr, et al., 2016). In the post-analytical phase, which comprises 18.5% of errors, an important and decisive tool to guarantee the patient's perfect therapeutic conduct is to report the reference values of the processed tests (Plebani, 2009), according to the laws in force in the country (Brasil, 2005). Likewise, in the area of scientific research, the data used as a reference are based on information provided in the information leaflet or in the old literature, not consistent with the reality of each research center or university (Dantas et al., 2008).

Given the importance of standardizing the physiological parameters of hepatic and

renal biomarkers involved in experimental models of scientific research, the present study aimed to establish the references of renal profiles being urea and creatinine and hepatic being alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in *Rattus norvegicus* kept in the maintenance vivarium of the University of Franca.

2. Material and Methods

For this, we used 169 animals, *Rattus norvegicus*, Wistar strain, young adults aged between five and 12 weeks, male, healthy, weighing between 150-310g, from the Central Vivarium of the University of São Paulo, Ribeirão Preto's Campus. The animals were transported to the Vivarium of the University of Franca, where they underwent a period of seven-day acclimatization with place and diet. The animals were kept during the whole experiment in the Vivarium of University of Franca, in polypropylene boxes with a double bed under controlled conditions of temperature (22º ± 2ºC), humidity (50 ± 10%) and illumination (light and dark cycle / 12 hours), with food and water ad libitum (projects approved by the Animal Ethics Commission of the University of Franca - No. 059/15, 6134300818 and 2655301116).

After anesthesia of the animals with sodium thiopental (840mg / kg / intraperitoneal / single dose), blood samples were collected by puncture of the left cardiac chamber using sterile hypodermic needle and syringe. Then, the aliquots were placed in microtubes without anticoagulants and sent to the Veterinary Clinical Analysis Laboratory of the University of Franca to be centrifuged at 3500 rpm for 15 minutes for serum separation and processed by the spectrophotometry technique, using the Labtest Kit in semi-analytical methodology in analysis on LabMax100 equipment for ALT and AST (U/L), urea and creatinine (mg/dL) dosages. For quality assurance, any sample that presented any degree of hemolysis was discarded.

From the obtained values, the statistical Rout test 1% test was applied to exclude the values considered outliers, aiming at a reliable result. For each serum parameter evaluated, mean ($\overline{x}$) and standard deviation (std) were expressed. Reference limits (percentiles) were determined as values found using the equation $\overline{x}$–(2*std) for lower values and $\overline{x}$+(2*std) for higher values, with two downward and upward deviations from the mean.

3. Results and Discussion

The mean±std values obtained were ALT 50 ± 19 U/L (n=100), AST 115 ± 36 U/L (n=127), Urea 46 ± 13 mg/dL (n=169) and Creatinine 0,50 ± 0,13 mg/dL (n=169), expressed in Table 1.

Table 1- Analytes evaluated for validation of reference results of *Rattus norvegicus* from the Central Vivarium of the University of São Paulo, Ribeirão Preto campus and other references.

Exame	Ref. 1	Ref. 2	Ref. 3	Ref. 4	Ref. 5	Ref. 6
ALT (U/L)	21 – 96	26 – 76	38 – 82	74 – 143	40 – 80	10-88*
AST (U/L)	44 – 186	58 – 104	61 – 210	18-45	80 – 125	11-39**
Urea (mg/dL)	20 – 73	33 – 63	26 – 58	12 – 25	34 – 70	15 - 40*
Creatinine (mg/dL)	0,23 – 0,77	0,36 – 0,64	0,24 – 1,20	0,20 – 0,50	0,15 – 0,85	0,5 – 1,5 *

1: Central Vivarium of the University of São Paulo, Ribeirão Preto campus;
2: Central Vivarium of the State University of Maringá (Dantas et al., 2008);
3: Central Vivarium of the University of Tiradentes (Lima et al., 2014);
4: Charles River Laboratory - Montreal;
5: Central Biottery of the Federal University of Ceará (Lima, 2018);
6: Labtest package insert * Labtest package insert according to Labtest Diagnostic Reagent for dogs, validated on LabMax100 rodent equipment. ** Label value according to Labtest in Reagent focused on human diagnostics, having its validation in LabMax100 rodent equipment.

Therefore, the reference values are shown in Table 1, together with those found in the literature and manufacturer's reagent package leaflet. From the data analysis it was possible to observe divergent alterations of the results found in this work. When compared to the literature, liver enzymes differ with the other authors, as already observed by Castello *et al*. (2011) who attributed this change to analytical methodologies performed, geometric location of animals, type of feed, handling, storage of samples, among others. These changes have also been described in other studies (Dantas et al., 2008; Melo et al., 2013; Lima et al., 2014); however, they do not present statistically significant differences by the Rout test 1% when compared the lower or higher values with each other.

Regarding the sample *n* for calculation of the reference value, there was divergence regarding the documents found in the literature, since the variation is between 30 to 700, emphasizing that the number of analytes performed by the laboratory may be high (Ferreira and Andriolo, 2008). Another relevant data is the definition of the person responsible for the procedure and validation of the reference values, which may be the technical director of the laboratory, according to Joint Commission on Accreditation of Healthcare Organizations (JCAHO, 1998) and College of American Pathologists (CAP, 1998).

Compared with the package leaflet values, the liver enzyme AST showed greater divergence, and it is important to emphasize that the package leaflet is developed for human

diagnostics, since in the market there is no availability for a veterinary kit (Lima et al., 2014). The relation between the alteration of reference values in rats in relation to AST compared to the package leaflet value can be interfered due to the collection by cardiac puncture (Dantas et al., 2008; Lima et al., 2014). Regarding renal profile, from the measurement of urea and creatinine, no statistically distinct alterations and package leaflet values were observed, being similar in all published studies (Dantas et al., 2008; Castello Branco, 2011; Lima et al., 2014).

The Clinical & Laboratory Standards Institute in document C24-A3 defined reference values as results for normal range measurement within a previously defined and controlled population, leading the clinician to understand changes when these values deviate from the normal range (CLSI, 2016). The same was described by Ferreira *et al.* (2008) when analyzing documents from the World Health Organization and International Federation of Clinical Chemistry and Medicine Laboratory emphasized the importance of obtaining their own reference values in assisting the assisted population.

In Brazil, the legislation responsible for the standardization of laboratories is the RDC 302 (Brasil, 2005) which emphasizes the importance of including reference values in laboratory reports issued (item 6.3.3 subitem *m*), however they do not indicate how to obtain such values, and managers should include them. Generally, the data are obtained by values provided by equipment validations or by the reagent kit package insert values (Ferreira &Andriolo, 2008).

Other important factors regarding the changes found in other articles in the literature are given as linearity of the reagent kit used and which equipment, and several authors did not disclose this information (Lima et al., 2014). Lima *et al*. (2014) cited that the comparison of results of analytes references in different methodologies may bring divergences. Still, other important factors to be observed within the pre-analytical phase is the diet in which the animal is submitted before the serum examination. Regarding the analytical phase, information such as material processing (manual, semi-automated or automated) as well as the reagent kit used, calibration and internal and external quality controls (Melo et al., 2013) are also relevant.

The determination of biochemical parameters in a local environment is necessary due to seasonality, different strains of animals because they work with heterogeneous animals.

4. Conclusion

The data found in this work are extremely important, due to the characterization and standardization of parameters of markers of liver and kidney lesions in an animal model used in scientific research of the Maintenance Vivarium of the University of Franca, helping research involving animals.

The characterization is necessary due to the difference between the reference values of other animal houses, corroborating with intrinsic (handling, applied methodology, maintenance, diet) and extrinsic (environmental origin, geographical location) factors and each research center should have their values. established to reduce the number of animals in trials.

This work is capable of alerting other research centers about the need to work with their own reference values for biochemical parameters of animals raised in local animal houses since the regionality, seasonality, equipment and reagent kits can be changed.

5. Acknowledgement

This work was carried out with the support of the Higher Education Personnel Improvement Coordination - Brazil (CAPES) - Financing Code 001. The University of Franca and FAPESP for the financial incentive. The team of the Veterinary Clinical Analysis Laboratory of the University of Franca.

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