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Effect of Different Routes of Blood Collection on the Results of Liver and Kidney Function Test of Adult Wistar Rats

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Abstract

Assessment of blood parameters is often used to monitor animal health and to determine the success of dietary and pharmacological intervention in preclinical animal models. It is important to consider whether hematological and metabolic measurements might differ in relation to different blood sampling sites at the time of blood collection. This research is aimed at evaluating the effects of the different routes of blood collection on the results of kidney function tests and liver function tests of adult Wistar rats. A total of 15 rats weighing 110g-150g were separated into three groups of 5 rats each and then allowed to acclimatize for 2 weeks. Blood was collected from group A using ocular puncture and from groups B and C through cardiac puncture and neck laceration mode of blood collection respectively. Liver function tests to determine serum levels of liver enzymes - aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and kidney function tests to determine the serum levels of urea and creatinine were carried out and the results recorded. Our results showed that the different routes of blood collection used have no effect on the serum level of AST, ALT, ALP, urea and creatinine. Hence, it is safe to say that the different routes of blood collection have no effect on the result of the kidney and liver function tests.

Keywords: Blood; Alanine Aminotransferase; Aspartate Aminotransferase; Liver Function; Kidney Function

Abbreviations: AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase

Introduction

One fundamental preanalytical procedure in laboratory testing is the collection of blood. Blood collection from small laboratory animals is necessary for a wide range of scientific research and, there are a number of efficient methods available for that [1-3]. Assessment of blood parameters is often used to monitor animal health and to determine the success of dietary and pharmacological intervention in preclinical animal models. More than one blood collection site may be used concurrently during a study, which would potentially confound the results [4].

Laboratory devices used for blood collection interact with blood to alter blood composition, serum or plasma fractions and in some cases adversely affect laboratory tests [5]. It is worthy of note that stress and other physiological reactions in rats during blood collection may have adverse impacts on the variables under study which may result in the invalidation of the results [6]. Therefore, it is important that blood sample collection from experimental animals be least stressful as stress might negatively affect the outcome of the study [7]. It becomes imperative to consider whether

hematological and metabolic measurements would differ in relation to different blood sampling sites at the time of blood collection.

Common considerations in the selection of sample techniques are their practicality and ease of use, the ability to attain the desired blood volume, sample quality and the impact on animal well-being [8]. The different routes of blood collection in experimental animals include blood collection not requiring anesthesia (saphenous vein, dorsal pedal vein), blood collection requiring anesthesia (tail vein, orbital sinus, jugular vein) and terminal procedures (cardiac puncture, posterior vena cava, axillary vessels, orbital sinus) as described by Hoff, et al. [9]. Each method can affect the outcome of serum biochemistry analysis, due to differences in handling, restraining, anaesthesia, invasiveness and animal discomfort [10,11].

Materials and Methods

Fifteen (15) adult Wistar rats weighing between 110g-150g were used for this study. They were randomly assigned into three groups (A, B and C) with five animals in each group. The rats were acclimatized for 2 weeks, housed in iron cages under standard laboratory and photoperiodic conditions, and fed with standard feed and water ad libitum.

Blood Collection and Clinical Chemistry Parameters

The rats were anaesthetized using chloroform sedation before the collection of blood. Blood was collected from rats in groups A, B and C through ocular puncture, cardiac puncture and neck laceration respectively. The blood collected was then put inside a plane bottle and spun in a centrifuge for 10 minutes at 20,000 rpm. Serum activity; Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), creatinine and urea were determined using an automated analyzer. Standard controls were run before each determination.

The blood sampling in this work was performed by the same skilled technician for all samples and all actions performed before and after blood collection were standardized, so that variability due to these reasons could be excluded from our results. Therefore, we assume that any difference in the values assessed reflects factors directly associated with the blood sampling method.

Statistical Analysis

Data obtained from this research were analyzed using SPSS version 23. The comparative analysis was done using ANOVA, and the level of significance was considered at $P < 0.05$.

Results

Results are presented as mean ± standard deviation (S.D) of 5 rats in each group; Statistical significance at $P<0.05$; A = Ocular puncture; B = Cardiac puncture; C = Jugular laceration; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase. Groups B and C were compared to group A in all the tables.

Kidney Function Test

Results are presented as Mean ± SD of 5 rats in each group. Results were considered significant at $p\leq0.05$.

The results in Table 1 above show no statistically significant difference in the blood urea and creatinine levels from rats in the three groups with different sources of blood collection.

Parameter	Group		
	A	B	C
Urea (mmol/L)	10.70 ± 3.38	10.63 ± 3.35	13.23 ± 4.27
p-value	-	0.4656	0.4537
Creatinine (umol/L)	180.47 ± 7.96	73.33 ± 9.94	84.60 ± 3.56
p-value	-	0.1.91	0.5972

A: blood collection by ocular puncture; B: blood collection by cardiac puncture; C: jugular laceration

Table 1: Result of Blood Urea.

Liver Function Test

Results are presented as Mean ± SD of 5 rats in each group. Results were considered significant at $p\leq0.05$.

The results obtained for the liver function parameters ALT, AST and ALP reported in Table 2 shows no significant difference in levels of these parameters in rats in the three group, notwithstanding the source of blood collection.

Parameter	Group		
	A	B	C
ALT (U/L)	14.30 ± 5.80	13.10 ± 3.06	12.63 ± 5.31
p-value	-	0.7322	0.9373
AST (U/L)	18.27 ± 5.14	19.90 ± 1.48	13.47 ± 4.73
p-value	-	0.628	0.4204
ALP (U/L)	92.00 ± 5.21	67.67 ± 4.61	105.57 ± 3.30
p-value	-	0.7947	0.2474

A: blood collection by ocular puncture; B: blood collection by cardiac puncture; C: jugular laceration; ALT: Alanine Aminotransferase; ASP: Aspartate Aminotransferase; ALP - Alkaline Phosphatase

Table 2: Result of Liver Function Test.

Discussion

Many sources of variation can affect the result of liver and kidney function biochemistry assay. These alterations can result from several factors such as events that occur prior to sample collection, such as fasting conditions or environmental stress. The blood sampling process itself is a source of variation, including the collection site, anesthesia used, level of hemolysis, and the skill of the technician performing the procedure [12]. Finally, factors during the period that follows blood collection may influence biochemical parameters and include tube selection, processing delay and the analytical procedure itself [11]. There have also been contentions as to the most correct route of blood collection, with many choosing ocular puncture as a superior method to others.

In light of the contentions as to the correct route of blood collection and the ability of the sampling method to alter biochemistry assay results, we focused on the effect cardiac puncture and jugular laceration blood collection methods have on liver and kidney function test results as compared to the effect ocular puncture blood collection method has on liver and kidney function test results. In table 1, it was observed that cardiac puncture and jugular laceration method of blood collection had no statistically significant difference in blood urea level as compared to that of ocular puncture. This result contradicts the work of Itziar, et al. [11] in which variations were seen in blood urea levels between the different routes of blood collection used. Also, the result of blood creatinine level shown in table 1 showed no significant statistical variation among the different routes of blood collection used (cardiac puncture and jugular laceration) as compared to ocular puncture.

It was observed that the different routes of blood collection used (cardiac puncture, jugular laceration and ocular puncture) had no significant effect on the result of alanine aminotransferase (ALT) as shown in (table 2). Alanine aminotransferase (ALT) is a liver-specific enzyme involved in the deamination of alanine. The observed values of ALT in the different methods of blood collection used corresponded with the recommended range of 7-56 U/L as reported by Diana [13].

According to the result as seen in table 2, we found no statistically significant difference between the three blood sampling techniques (cardiac puncture, jugular laceration and ocular puncture) for the result of aspartate aminotransferase (AST). The AST values observed in our study were in line with the recommended range of 0 to 35 U/L as reported by Diana [13]. The result for alkaline phosphatase (ALP) showed no significant difference in the value associated with the cardiac puncture and jugular laceration method of blood collection as compared to the ocular puncture method of blood collection. The values observed coincide with the range of normal alkaline phosphatase level of 41 to 133U/L given by Diana [13,14].

Conclusion

Different routes of blood collection have no statistically significant effect on the results of liver and kidney function tests as against many believe that blood collected from different routes gives different results and may affect the outcome of research.

Ethical Approval

Ethical approval was obtained from the Animal Ethical Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi.

Conflict of Interest

Authors declare no conflict of interest.

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