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Article

Stability of Aspartate Transaminase in Blood Specimens Subjected to Various Storage Temperatures and Times Prior to Centrifugation

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Abstract

Background: Aspartate Transaminase (AST) is an enzyme used to diagnose medical problems and found in erythrocytes other than in the heart, liver, skeletal muscles, kidney, and brain. Prolonged serum-clot contact time and the fluctuation in storage temperature are possible reasons for the changes in chemical constituents in serum. The objective of this experimental study was to determine the effect of the storage time (three and six hours) before centrifugation and the storage temperature (4°C and 28°C) on the stability of AST in blood. **Methods:** The study sample consisted of fifty-three healthy adult volunteers. Five milliliters of blood samples were collected from each subject and then aliquoted into five Eppendorf tubes. One of the aliquots was centrifuged within 30 minutes of collection, and the AST assay was done to obtain the control (baseline) value. Two of the remaining aliquots were stored at 4°C while the other two aliquots were stored at room temperature for three hours and six hours, respectively prior to centrifugation. The difference between the baseline AST values and paired values of samples at different times of storage and temperatures was analyzed using the non-parametric Wilcoxon signed-rank test. **Results:** Significant differences were found in AST level compared to the baseline value when samples were stored at room temperature for three hours ($p=0.027$) and six hours at room temperature ($p=0.011$). **Conclusions:** AST in blood was unstable for at least three hours at room temperature (28°C). At 4°C, AST was stable for up to six hours, and blood samples can be stored at 4°C for up to six hours for analysis of AST.

Keywords: Aspartate transaminase or aminotransferase, AST, Room temperature

Introduction

Aspartate transaminase (AST) is a biological catalyst mainly found in the liver and is elaborated by extrahepatic tissues including muscle tissue, heart cells, pancreas, kidneys, and red blood cells (1,2). AST is a transaminase enzyme, also known as serum glutamate-oxaloacetate transaminase (SGOT). It catalyzes the reversible transfer of an amino group from Aspartate to α-ketoglutarate and the products of this transamination reaction are oxaloacetate and glutamate (3). AST level gives useful information about a patient's liver and heart damage and it may also indicate other health issues (4). Even though the measurement of AST activity aids primarily in the diagnosis of liver diseases, it is not liver-specific. However, the AST levels could be used along with the other enzymes in monitoring the causes of various liver disorders. Elevated AST levels can be found when the additional enzyme is released into the

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bloodstream due to tissue or organs such as the heart or liver being damaged or diseased. An increase in AST enzyme levels to about 10 to 20 times indicates severe tissue damage, and the ratio of AST to alanine transaminase (ALT) is used in determining the disease conditions (1).

Maintaining of integrity and reliability of laboratory results is one of the major challenges faced by clinical laboratories worldwide. Pre-analytical errors have become a dominant component in the variability of laboratory results even with the development of new technologies. When the unseparated samples are kept at ambient temperature, many blood analytes can deteriorate within hours. Therefore, it is important to carry out the test as soon as possible (5). If not possible the separated sample should be refrigerated and analyzed within 24 hours (6). However, most of the time in resource limited hospitals these blood specimens have to be stored for a longer time before centrifugation due to practical constraints such as inadequate facilities available in the peripheral laboratories, delayed transportation, power failure, and improper collection and handling procedures (5). In peripheral laboratories of Sri Lanka blood samples are collected from individuals and transported them to the central laboratories. It may take time to transport these blood samples to central laboratories from varying distances under various storage conditions and transportation modes. In the case of a power failure, the blood samples may be kept at room temperature before separation of serum by centrifugation until they are processed.

When storing blood samples for several hours at room temperature, some constituents of serum can be changed into values that falsely indicate clinical significance (7). Prolonged serum-clot contact time and the fluctuation in storage temperature are possible reasons for the changes in chemical constituents in serum. However, limited information is available regarding the stability of AST in blood specimens subjected to various storage temperatures and time durations prior to centrifugation.

The objective of this experimental study was to determine the effect of storage time (three and six hours) before centrifugation and the storage temperature (4 °C and 28 °C) on the stability of AST in blood. Therefore, the study investigated the change in AST level of blood samples kept at refrigerator (2-8 °C) and room temperature (28 °C) for up to 6 hours.

Materials and Methods

The venous blood samples were collected from randomly selected 53 healthy adult volunteers. The volunteers were not on anticoagulant therapy, not known to be anemic and did not undergo a regular phlebotomy for any reason. Approximately 5 ml of blood was collected into labeled red-top vacutainer specimen tubes from each volunteer by venipuncture after the written consent. Each sample was aliquoted into five Eppendorf tubes numbered from one to five containing 1ml of blood. All Eppendorf tubes were kept at room temperature for at least 30 minutes for clot formation prior to the centrifugation.

Aliquot No:1 from all the samples was centrifuged at 3000 rpm for ten minutes and used to determine the baseline value of the AST level. To determine the effect of storage temperature and time on the AST level of non-centrifuged blood samples, they were treated as follows: Aliquot No: 2 was kept at the room temperature range of 28 – 30 °C (average 28 °C) for three hours, Aliquot No: 3 was kept at refrigerator at 4 °C for three hours, Aliquot No: 4 was kept at room temperature for six hours, and Aliquot No: 5 was kept at refrigerator at 4 °C for six hours. At the end of storage time with different temperatures, samples were centrifuged at 3000 rpm for 10 minutes, and serum was separated. AST level was measured in each sample by the modified method of the International Federation of Clinical Chemistry for AST (Biolabo, France) in KONE 20 XT Auto analyzer (Thermo Scientific, Finland). All analyses were done on duplicate samples each time, and the average of the two results was recorded.

The study was approved by the Ethics Review Committees of the Faculty of Medical Sciences, University of Sri Jayewardenepura. Written informed consent was obtained after explaining the purpose of the study to each volunteer prior to the commencement of the study.

The results obtained from the samples stored in a refrigerator (4 °C) were also compared with those obtained from samples kept at room temperature using the Wilcoxon signed-rank test. Also, the sample results stored at three hours and six hours were compared with each other using the Wilcoxon test. The results were evaluated at 95% confidence level, and results were expressed as median (IQR) and p value ≤ 0.05 was considered to be statistically significant.

Results

This study was designed to evaluate the effect of storage time and temperature on stability of AST in blood. The mean AST values of samples stored for zero hour, three hours and six hours at room temperature (28-30 °C) were 28.9 (9.9) IU/L, 30.1(8.7) IU/L and 29.6 (9.4) IU/L, respectively (Table 1). The mean AST values of samples stored for 3 and 6 hours at 4 °Cwere 28.9 (9.2) IU/L and 29.9 (9.7) IU/L, respectively (Table 2).

The mean AST values of samples stored for 3 hours and 6 hours at room temperature showed significant differences (p=0.027, p=0.011), respectively when compared with the baseline value (at zero hour) (Table 1). However, there was no statistically significant difference (p=0.275, p=0.228) between the mean AST values when samples were stored for 3 and 6 hours at 4°C with baseline value (Table 2).

Table 1. The influence of storage time at room temperature on AST level

Storage time at room temperature (hours)	AST level median (IQR) (IU/L)	p value
Zero hour	28.9 (9.9)	
3 hours	30.1 (8.7)	0.027
6 hours	29.6 (9.4)	0.011

Table 2. Influence of storage time at 4°C on AST level

Storage time at 4 °C (hours)	AST level median (IQR) (IU/L)	p value
Zero hour (Baseline)	28.9 (9.9)	-
3 hours	28.9 (9.2)	0.275
6 hours	29.9 (9.7)	0.228

Discussion

The Clinical and Laboratory Standards Institute guidelines state that serum samples should be separated from cells within 2 h of collection for most of the analytes. It also states that temperature may affect the stability of some analytes (8). The goal of this study was to determine the effect of storage time (three and six hours) before centrifugation and storage temperature (4 °C and 28 °C) of blood samples on the stability of AST in blood.

In the current study we found a percentage increase in mean AST value with storage time. According to that AST level was increased by 2.28% and 4.20% when samples were stored for three and six hours at room temperature, respectively. This finding is supported by the previous study which showed that the percentage value was slightly increased with time (9). In the current study there was a statistically significant increase in AST level if the blood samples were kept at room temperature (28-30 °C) for 3 hours (p=0.027) and 6 hours (p=0.011) when compared to control (baseline value). Ono *et al*. reported a significant increase in AST level when the storage time was as long as 6 hours at 30°C and a significant

increase was not observed when samples were stored for 3 hours at 30 °C (10). However, a statistically significant increase was observed at room temperature when samples were stored for 3 hours, which was a new finding reported in this study.

In the current study statistically significant differences (p=0.275, p=0.228) were not observed in mean AST values when samples were stored for 3 and 6 hours at 4 °C. This finding was consistent with the results of previous studies (10,11). According to our results, the reason for this significant increase in AST level may be due to the AST movement from the cellular compartment to the serum due to changes in cell permeability and fragility of the cell membrane (7). When there is prolonged contact of red blood cells with serum, it is possible that a leakage of water into the red cells due to failure of Na^+/K^+ pump in the cell membrane to maintain osmotic balance resulting in swelling of red cells, increased hematocrit, decreased serum volume leading to increase the AST concentration (12).

This study revealed that the maximum storage time of blood samples prior to centrifugation at room temperature should be less than three hours from phlebotomy if AST levels need to be determined. Our study has already proved that it is possible to store blood samples in the refrigerator (4 °C) for up to 6 hours of blood collection, as there were no significant changes in results.

Conclusion

In conclusion, AST is not stable in blood when samples are stored for at least 3 hours at the room temperature range of 28 °C -30 °C. However, AST in blood is stable up to six hours from phlebotomy if stored at 4°C. Therefore, blood samples can be stored at 4 °C for up to six hours for the analysis of AST.

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Conflict of Interest: The authors declare no conflict of interest.

References

1. Huang XJ, Choi YK, Im HS, et al. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors. 2006, 6(7): 756-82.
2. Limdi J, Hyde G. Evaluation of abnormal liver function tests. Postgrad Med J. 2003, 79(932): 307-12.
3. Kamitori S, Okamoto A, Hirotsu K, et al. Three-dimensional structures of aspartate aminotransferase from *Escherichia coli* and its mutant enzyme at 2.5 Å resolution. J Biochem. 1990, 108(2): 175-84.
4. Yap CY, Aw TC. Liver function tests (LFTs). Proc Singapore Healthc.. 2010, 19(1): 80-2.
5. Kachhawa K, Kachhawa P, Varma M, et al. Study of the stability of various biochemical analytes in samples stored at different predefined storage conditions at an accredited laboratory of India. J Lab Physicians. 2017, 9(01): 011-5.
6. Bermes E, Young DS. General Laboratory Techniques. Tietz Text Book of Clinical Chemistry Burtis, CA and Ashwood ER (Eds), 3rd ed Saunders Company, Philadelphia, USA. 1999.
7. Rehak NN, Chiang B. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem. 1988, 34(10): 2111-4.
8. CLSI document H 18-A3. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline. 3rd edn. 2004.

9. Laessig RH, Indriksons AA, Hassemer DJ, et al. Changes in serum chemical values as a result of prolonged contact with the clot. Am J Clin Pathol. 1976;66(3):598-604.
10. Ono T, Kitaguchi K, Takehara M, et al. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. Clin Chem. 1981;27(1):35-8.
11. Tanner M, Kent N, Smith B, Fletcher S and Lewer M. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Ann Clin Biochem. 2008, 45: 375–79.
12. Clark S, Youngman LD, Palmer A, et al. Stability of plasma analytes after delayed separation of whole blood: implications for epidemiological studies. Int J Epidemiol. 2003, 32(1): 125-30.