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ORIGINAL ARTICLE

Effect of Storage Temperature on D-Dimer Stability in Different ABO Blood Groups

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Abstract

Background: D-dimer is the end product of fibrin degradation formed during the disintegration of blood clots and fibrin networks. It is an invaluable analyte in evaluating thrombin and plasmin activity and is specific for fibrin derivatives. This study evaluated the effects of storage temperatures on D-dimer stability in different ABO blood types stored over a defined period of time.

Materials and Methods: The study was performed with 40 samples collected randomly from apparently healthy male and female undergraduate students of Rivers State University, Port-Harcourt, aged between 17 and 45 years. Two milliliters (2ml) of whole blood were collected from each participant with the specified ABO blood type into ethylene diamine tetra acetic acid (EDTA) anticoagulant bottle, and plasma was separated and stored at two different temperatures of +6^{o}Cand +25^{o}C for a period of 24 hours and 48 hours respectively. Following the manufacturer's instructions, the plasma blood sample was analyzed using the Standard F-D-dimer FIA reagent and SD-Biosensor analyzer. Data obtained were analyzed statistically using the Statistical Package for Social Science (SPSS) version 23, and results were considered significant at $p< 0.05$.

Results: Results obtained showed that D-dimer baseline values vary significantly across the various blood groups O (2120.50±0.71), followed by A (411.00±1.41), AB (167.00±1.41) and B (149.50± 0.71) respectively. D-dimer values decreased after storage at +6oc for 24 hours in group A (324.50 ± 0.71), AB (149.50±0.07), and O (1499.00±1.41) but increased in B (151.00 ± 1.41) when compared with baseline values ($p<0.05$). At +25^{o}C, D-dimer values increased in blood group A (443.00 ± 1.41), and B (155.001±1.21) and decreased in AB (154.50 ± 0.71) and O (1649.00 ± 1.41) after 24 hours storage but increased in B and AB while decreasing in A and O after 48 hours storage ($p<0.05$) respectively.

Conclusion: This study revealed that D-dimer levels vary based on different blood groups, prolonged blood storage affects D-dimer stability, and different storage temperatures have discernable impacts on D-dimer stability. Thus, blood samples for the D-dimer test should be assayed immediately after sample collection and

separation and not stored to avoid erroneous results.
Keywords: D-dimer, ABO Blood types, Storage Temperature.

Introduction

The measurement of D-dimer levels remains a critical tool in diagnosing coagulation disorders and assessing thrombotic risk, but the impact of storage temperature and duration of storage on the stability of D-dimer analysis within blood samples remains a subject of concern. D-dimer has emerged as a pivotal parameter for evaluating various disorders, particularly those intertwined with coagulation and fibrinolysis (1).

D-D-dimer is a term that denotes multiple peptide fragments resulting from the plasmin-mediated degradation of cross-linked fibrin (the end product of fibrin degradation) formed due to the breakdown of blood clots and fibrin networks. It holds a significant utility as a biomarker for diagnosing and monitoring thrombotic and fibrinolytic processes and signifying concurrent activation of coagulation and fibrinolysis (2, 3, 4).

The generation of D-dimer involves three enzymes: thrombin, activated factor XIII (factor XIIIa), and plasmin (2). the process begins as thrombin, produced by the coagulation system, transforms soluble fibrinogen into fibrin monomers that subsequently assemble themselves into fibrin polymers through noncovalent interventions as controlled by allosteric changes within the proteins mainly triggered by thrombin cleavage of fibrinopeptide from the N-terminal domain (2, 3). The process initiates as thrombin, produced by the coagulation system, transforms soluble fibrinogen into fibrin monomers. These monomers subsequently assemble into fibrin polymers through non-covalent interactions driven by allosteric changes within the protein, triggered by thrombin cleavage of fibrinopeptides from the N-terminal. This will activate Factor XIII (fibrin stabilizing factor), reinforcing the fibrin through the D-domains of neighboring fibrin monomers. Then, plasmin acts on the fibrin clot, forming the D-dimer molecule (3, 5).

The stability of D-dimer within blood samples during storage constitutes a paramount concern in the sphere of clinical laboratories and medical research. To ensure the precision and reliability of D-dimer measurements, meticulous sample collection and handling and appropriate storage conditions must be deployed at all times (6), as deviations from the recommended storage conditions can precipitate D-dimer degradation and potentially yield inaccurate findings. Few researchers have suggested that factors such as temperature, duration of storage, and composition of the blood sample can influence D-dimer stability (7, 8), but there are still varying and divergent views on this, and thus, this is needed for this research.

The ABO blood group system is a categorization of blood based on the presence or absence of specific antigens on the surface of red blood cells (9). The ABO, followed closely by the Rhesus (Rh) system, is highly immunogenic and has gained extensive attention in medical research due to its clinical implications in transfusion medicine and susceptibility to various diseases (10, 11, 12, 13). The influence of ABO blood groups on an array of physiological and pathological processes, such as haemostasis and thrombosis, is well-established, with reports of discernible disparities in haemostatic parameters and coagulation profiles among individuals bearing different ABO blood groups (7, 13, 14). This study evaluated the effects of storage temperatures on D-dimer stability in different ABO blood types stored over a defined period of time.

Materials and Methods

Study Design and Population

This study is a cross-sectional study carried out

on stored blood collected from undergraduate students of Rivers State University, Port-Harcourt. The study was performed with 40 samples collected randomly from apparently healthy male and female undergraduate students of Rivers State University, Port-Harcourt, with the blood groups A, B, AB, and O, all aged between 17 and 45 years and who willingly gave consent as participants in the study.

Sample Collection

Venous blood was collected into a sample bottle containing 0.5 ml of 12mg/ml of dipotassium ethylene diamine tetra-acetic acid (EDTA) and well mixed to determine D-dimer.

Two milliliters (2ml) of whole blood were aseptically collected from each participant with the specified ABO blood type and into a sample anticoagulant bottle containing 0.5ml of 12mg/ml of ethylene diamine tetra acetic acid (EDTA) and plasma separated after a gentle spin, and stored at two different temperature of +6^{o}c and +25^{o}c for a period of 24 hours and 48 hours respectively.

Methodology: Determination of D-dimer using SD- Biosensor Autoanalyser

Following the manufacturer's instructions, the plasma blood sample was analyzed using the Standard F-D-dimer FIA reagent and SD-Biosensor analyzer.

Data Analysis

Data collected was analyzed using a statistical package for social sciences (SPSS). Version 23 was used for data analysis. Statistical tools such as mean and standard derivation (SD) were used. Analysis of Variation (ANOVA) was used to compare means of more than two groups.

Results

Demographic Details of Studied Population

A total number of 40 subjects were recruited for the study,10 from each ABO blood group (A, B, AB ,O), consisting of 15 male subjects and 25 female subjects all within the ages of 17-45 years, as shown in Table 1

Table 1 Demographic Details of Studied Population

Characteristics of the Population	Frequency	Percentage (%)
Age (years		
15-24	25	62.5%
25-34	10	25%
35-44	3	7.5%
45-54	2	5%
ABO Blood Groups		
A	10	25%
B	10	25%
AB	10	25%
O	10	25%
Sex/Gender		
Males	15	37.5
Females	25	62.5

Comparison of Parameters According to Blood Groups

Results of D-dimer values in the studied population across the various ABO blood group (A, B,

AB, O) is presented in Table 1. D-dimer values in groups A, B, AB, and O show statistically significant variation based on storage temperature ($p < 0.001$), as presented in Table 2, with blood group O being the highest at baseline and group B having the lowest baseline values.

Table 2 Comparison of D-dimer Values based on Storage Temperature in the study population

Measured parameter	D-Dimer			
Storage Duration/ Temperature	Group A	Group B	Group AB	Group O
Baseline	411.00±1.41[a]	149.50±0.77[a]	167.00±1.41[a]	2120.50±0.71[a]
24hours At 60C	324.50 ± 0.71[b]	151.00 ± 1.41[a,b]	149.50 ± 0.07[b]	1499.00 ± 1.41[b]
48hours At 60C	267.00 ± 1.40[d]	186.00 ± 1.41[c]	184.00 ± 1.41[d]	1314.50 ± 0.71[d]
24hours At 250C	443.00 ± 1.41[c]	155.001±1.21[b]	154.50 ± 0.71[c]	1649.00 ± 1.41[c]
48hours At 250C	288.00 ± 1.14[e]	165.00 ± 1.00[e]	186.00 ± 1.41[d]	1224.00 ± 1.41[e]
p-value	< 0.001	< 0.001	< 0.001	< 0.001
F-value	6790.235	267.294	394.750	1781.541
Remarks	S	S	S	S

Key: S= significant, Values with different superscripts (a, b, c, d, e) are significantly different from each other ($p<0.05$)

Discussion

The D-dimer assay evaluates thrombosis and plasmin activity and is specific for fibrin derivatives. D-dimers are formed from the breakdown of intravascular fibrin and serve as a marker of fibrinolytic activity.

Results obtained showed that D-dimer baseline values vary significantly across the various blood groups and are highest in blood group O subjects, followed by A, AB, and B, respectively. This implies that D-dimer values are higher in blood group O individuals and suggestive of the high fibrin breakdown/ fibrinolytic activity rate in blood group O individuals compared to the A, B, and AB, respectively. This might be due to certain genetic and biochemical factors related to their ABO blood group composition. The lack of antigens A and B on the surface membrane of group O individuals, the less expression of coagulation factors, and the absence or lack of transferase enzymes that add carbohydrates to H substance in A, B blood types lacking in blood group O individuals, thus providing grounds for the activities of coagulation in blood group O individuals can be a major source of this variations.

Also, it is known from research (15) that blood group O individuals have a low tendency to express coagulation factors such as von Willebrand factor and factor VIII due to lack of terminal carbohydrate (sugar); thus, blood group A and B have more von Willebrand factor and factor VIII than O and thus more chances of thrombotic activities. The finding in this study is in tandem with the observation of Raja et al. (16), who opined that D-dimer is associated with ABO blood groups, Böhm-Weigert et al. (7), who reported that it is plausible that D-dimer stability may also be

subject to modulation by inherent blood group variations. However, the findings in this study are in contrast with the study by Karakece et al. (6), who reported no significant difference in D-dimer stability in blood stored at three different temperatures and at eight different time intervals.

Dimer values decreased after storage at +6°c for 24 hours in groups A, AB, and O but increased in B when compared with baseline values ($p<0.05$). This implies that temperature and duration of storage significantly affect D-dimer's stability under storage conditions. These findings can be due to the degradation of certain proteins or factors that influence the measurement of D-dimer. The decrease in blood group O could also be due to the absence of antigens in the cell, which could make the breakdown of fibrin cloth by plasminogen easier.

At +25°C, D-dimer values increased in blood groups A and B and decreased in AB and O after 24 hours of storage but increased in B and AB while decreasing in A and O after 48 hours of storage ($p<0.05$), respectively. This suggests the impact of storage duration and temperature on the stability of D-dimer. As explained earlier, the structural differences and composition of these blood types also contribute to this variation in D-dimer level. This instability is chiefly sponsored by prolonged storage resulting in altered composition, subsequent degradation of fibrin clots, and release of end products of fibrinolysis (D-dimer). Also, these changes in D-dimer could result from a combination of protein degradation, interactions, and alterations in the clotting cascade unique to blood group AB, which might follow a distinct pattern during storage compared to other blood types. Furthermore, temperature fluctuations, other environmental factors, the biochemical nature of the blood group, and anticoagulant interference/ activities could have also interfered at their peak in this storage duration and temperature, accounting for the variations recorded. A potential reason for the disparity in results in this study could be that the study was conducted with different blood groups, differing from other studies where D-dimer stability was evaluated on whole blood and stored blood at different time intervals and temperatures without considering variations in blood groups.

Conclusion

This study revealed that D-dimer levels vary based on different ABO blood groups, prolonged storage of blood affects D-dimer stability, and different storage temperatures have discernable impacts on D-dimer stability.

Recommendation

Blood samples for the D-dimer test should be assayed immediately after collection and separation and not stored to avoid erroneous results. D-dimer values are more stable in blood samples stored at room temperature than at 6°C.

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