

The Difference of Plasma Prothrombin Time Value in the Production of Adsorbed Plasma Using Various Concentrations

by Paulus Budiono Notopuro

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The Difference of Plasma Prothrombin Time Value in the Production of Adsorbed Plasma Using Various Concentrations

of Barium Sulphate in D1'. Soetomo General Hospital, Surabaya, Indonesia

Put11 Devi Oktapiani Putril, Paulus Budiono Notopuroz

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Abstract

Detection of a factor deficiency in the case of coagulation prolongation is very important. In this case a factor deficient plasma is required. This research provides a simpler method to produce factor deficient plasma, using barium sulphate as an adsorbent with Prothrombin Time (PT) of more than 60 seconds as a target. The aim of this study is to analyze the difference of PT value in various concentrations of barium sulphate. Pooled normal plasma (PDTPJ) was made from citrated blood and basic PT value was measured. Nine plain tubes were prepared with 1 mL of PT for each tube. Barium sulphate 200-1000 mg/mL plasma was then added to each tube. Finally, PT value of each tube was measured. The presence of barium sulphate <800 mg/mL plasma made no significant changes in PT. Conversely, the presence of 2800 mg/mL plasma increased PT more than 5 times compared to normal PT. Adsorbed plasma lacks vitamin K dependent factors because barium sulphate binds to calcium polypeptide, and removes all vitamin K dependent factors. The barium sulphate concentration used to make adsorbed plasma in this research was different with previous research. It was found that barium sulphate with 2800 mg/mL plasma can significantly change the PT value and effectively reach the PT target of adsorbed plasma as a basic reagent for substitution study. In conclusion, different concentrations of barium sulphate used resulted in different PT values. Optimal concentration of barium sulphate to make adsorbed plasma was 2800 mg/mL plasma.

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Introduction

Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) examinations are routine

tests performed to evaluate coagulation function. There are more than 40,000 PPT and APTT examinations at RSUD Dr. Soetomo per year with results that extend by 28%. In order to determine whether the coagulation abnormality is caused by a lack of coagulation factors or

Corresponding author:

Put11 Devi OktapianiPut1'i

Email: dev.i.o1(tapiani92@gmail.co111

the presence of a inhibitor. a mixing test with normal pooled plasma can be performed. If a coagulation factor deficiency is found, a substitution test can be performed to identify the deficient coagulation factor.

Currently, several coagulation factor examinations

are available, including the examination of VIII factor and IX factor. Both of these tests can provide information on levels of VIII factor and IX factor; however, these tests are quite expensive and cannot be performed in standard laboratories. The substitution test is a promising alternative as a method of examining coagulation factors. To perform a substitution test, two types of plasma are

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required, namely aged plasma and adsorbed plasma. The availability of these two types of plasma considerably supports the diagnosis of a coagulation disorder, even in a standard laboratory.

Preparation of adsorbed plasma requires a standard

method so that the adsorbed plasma obtained can be used in the substitution test. In order to produce adsorbed plasma, citrated blood plasma can be mixed with barium sulphate to achieve a predetermined PT value. This research examines the concentrations of barium sulphate that can be used for the manufacture of adsorbed plasma with citrated blood plasma.

Method

This research was conducted using cross-sectional

analysis. The samples of this study were collected from veins of Clinical Pathology Resident, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Hospital, Surabaya, Indonesia. Random sampling was done until 20 samples were obtained. This research was conducted in the Clinical Pathology Laboratory, Integrated Diagnostic Center Building.

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The inclusion criteria were samples from Dr. Soetomo General Hospital, Surabaya, Indonesia which were

accommodated in citrate anticoagulant tubes with volume in accordance with the minimum limit of citrate tube filling (2.7 mL). Exclusion criteria were samples with insufficient volume, clotted blood samples, icteric, lipemic, or hemolyzed samples. Independent T-test was performed to analyze the effect of difference of barium sulphate on PPT values. p value <0.05 was considered statistically significant. Data was analyzed using MS excel and SPSS software.

Results and Discussion

The preparation of adsorbed plasma in this study

used several variations of barium sulphate concentration. An increase in the concentration of barium sulphate can affect the PT plasma value produced. A very sharp increase in the value of PT was found in the plasma with 900 mg/mL of barium sulphate. With barium sulphate concentration of 1000 mg, the PT value that appeared on the device was no longer visible. In this case, the upper limit of the PT value that could be detected by the device was used, namely 130 seconds (Figure 1).

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Radar barium sulfat (mg/ml plasma)

Figure 1. The value of PT in the preparation of adsorbed plasma with various concentrations of barium sulphate.

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In this research, several cut-offs of barium sulphate

concentrations were chosen to observe how much change in the value of PT would occur when higher concentrations of barium sulphate were used or vice versa. In Table 1, it can be seen that there are 3 cut-offs, namely 600 mg/mL of plasma, 700 mg/mL of plasma, and 800 mg/mL of plasma. A difference test

was performed on each cut-off mean value of PT that could be achieved. In the use of barium sulphate <600 mg/mL of plasma, the mean value of PT was only 11.48 seconds with p value of 0.49, which was not statistically significant. When barium sulphate was used at 800 mg/mL of plasma or above, the PT value was statistically significant reaching 86.77 seconds.

Table 1. The Difference of PT Values with Barium Sulphate Concentration Grouped based on Cutoffs.

Concentration of Barium Sulphate Mean Value of PT (s)

Concentration (mg/mL of plasma) (seconds) P

<600 11.48

0.49
2600 61.90

<700 13.54

0.29
2700 72.52

<800 15.23

0.03
23800 86.77

The use of barium sulphate in the preparation of

adsorbed plasma has been investigated for decades. Hydrophobic materials such as barium sulphate and aluminum hydroxide can absorb certain coagulation factors (especially II, VII, IX, and X factors) when added to plasma. Other studies have shown that the use of aluminum hydroxide can reduce the activity of II, VII, IX, and X factors. Increasing the concentration of aluminum hydroxide used will further reduce the activity of these coagulation factors. Barium sulphate and plasma citrate was used in this research for the preparation of adsorbed plasma. The greater the concentration of barium sulphate used, the higher the PPT value. This suggests the possibility that the coagulation factors involved in the extrinsic coagulation pathway experience decreased activity.

Vitamin K-dependent coagulation factors have

one special feature, which is the fact that they have the same polypeptide structure. In Nelsestuen and Suttie's research, it was found that there was a structure called

o. peptide in prothrombin. This structure disappears after adsorption process. This peptide is a vitamin K dependent structure, and is present in all vitamin K dependent proteins.

Adsorption process caused a decrease in the

activity of II, VII, IX, and X factors. This study used aluminum hydroxide in gel form as an adsorbent. Each factor showed a different decrease in activity after the adsorption process. This was also influenced by differences in temperature and pH.

A literature stated that the concentration of barium

sulphate used for the preparation of adsorbed plasma is 100 mg/mL of plasma. A different literature stated that 33 grams of barium sulphate is mixed with 150 ml of plasma, to which a buffer solution has been added, then stirred at room temperature for 30 minutes. In this study, the use of barium sulphate below 600 mg/mL of plasma did not significantly increase the PT value. The use of barium sulphate at concentration of 600 mg/mL plasma

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produced plasma with PT value of 19.4 seconds (Figure 1). There are several variations in the target value of PT in the preparation of adsorbed plasma, namely 2/3 times the normal PT value, or PT >60 seconds. If the upper

limit of normal PT value is 13 seconds, then the use of

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plasma would succeed in achieving the minimum target value of PPT, namely 32.5 seconds.

Several cut-offs were selected to determine the

appropriate minimum concentration of barium sulphate in the preparation of adsorbed plasma. Based on the PT values achieved with the use of different concentrations of barium sulphate (Figure 1), several cutoffs for barium sulphate concentrations were taken as a reference to achieve the targeted PT value. Using the most minimum concentration of barium sulphate with the most effective result is very important for the efficiency of materials and processing time.

Table 1 shows some of the cutoffs that can be

considered as minimal concentrations that can be used for the preparation of barium sulphate. If a lower barium sulphate concentration could achieve the targeted PT value, it would certainly increase the efficiency and effectiveness of the process in making adsorbed plasma. Barium sulphate levels <800 mg did not reach the targeted PT value (Figure 1), and statistically also did not provide a significant difference in PT values (Table 1). Barium sulphate concentration of 2800 mg/mL of plasma resulted in the most statistically significant p value (p 0.03) with a PPT value that reached 34.3 seconds.

Conclusion

The use of barium sulphate with different

concentrations produce plasma with different PT values. Higher levels of barium sulphate increase the plasma PPT value, however the increase is non-linear. Barium sulphate with concentration of 2800 mg/mL is the most optimal for the preparation of adsorbed plasma as a basic reagent for substitution tests.

the manuscript.

Ethical Approval: The research ethic has been

8 approved by the Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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