ORIGINAL ARTICLE

Kappa Test With Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP) Blood Preparation Method for Examining the Value of Activated Partial Tromboplastin Time (APTT) and Plasma Protrombin Time (PPT)

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ABSTRACT

Examining the Activated Partial Thromboplastin (APTT) and Plasma Protrombin Time (PPT) is sort series of examining homeostasis which is conducted in order to have screening test for homeostasis disorder. This examination used plasma sample in which solidification factors were found which could be influenced by thrombocyte existence. The centrifuging of citrate blood sample which was conducted too fast or too slow would cause plasma conditions with the number of thrombocytes. Practical in some laboratories were not yet been uniformed, mainly in centrifuging of citrate blood to obtain citrate plasma with fewer thrombocyte contents. To identify the value of Kappa, it is by compatibility test between two methods PRP and PPP to examine PPT and APTT. This is true experimentalstudy with post-test research design without control. The citrate plasma sample was obtained from 10 samples of students' blood which had one pair, 2 treatments; by centrifuging 3000 rpm for 10 minutes for PPP and centrifuging 1000 rpm for 10 minutes for PRP. Then, both methods (PPP and PRP) were examined by using PPT and APTT parameter. In the result of PPT examination in the sample of PRP plasma, the average value was 11.6 seconds. In the sample of PPP, the average value was 11.0 seconds. The result of APTT examination in PRP sample, the average value was 34.27 seconds while in PPP sample was 33.18 seconds. There was compatibility in the result, either PPP method and PRP for PPT and APTT examination (Kappa = 1).
Keywords: PRP, PPP, APTT, PPT, Kappa.

INTRODUCTION

Hemostasis is the body's mechanism to stop bleeding spontaneously. Hemostasis is supported by several systems, namely the vascular system, platelets, and blood clots. Hemostasis test is usually conducted before surgery. Some clinicians require examination of hemostasis for all preoperative sufferers, but there are also those that limit it only to patients with a history of hemostatic disorders. Some types of hemostasis filter tests are Bleeding Time (BT), platelet count, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Thrombin Time (TT)[1].

Factors that need to be considered in APTT examination are the comparison of Na-citrate as an anticoagulant and the duration of blood-citrate dwelling (Jones and Wickramasinghe, 1995). In addition, the plasma for APTT examination is Platelet-Poor Plasma (PPP). PPP is plasma with a platelet count of <10,000 / mm3. The making of PPP is very much influenced by the speed and time of centrifugation. According to the Clinical and Laboratory Standard Institute (CLSI), PPP is obtained by centrifugation at a speed of 2000 g (3500 rpm) for 10 minutes or at a lower speed for 10-30 minutes[2].

Hemostasis test, especially for PPT and APTT, is strongly influenced by pre-analytic factors when preparing plasma citrate. There are factors that have been considered since the way of blood collection, dosage and mixing of citric blood, compaction, delivery, and storage of samples. There are also Analytical factors of inspection techniques, tools or instrumentation, tool calibration. For distillation of citrate blood samples, until today, there has been no uniformity of each laboratory about how long and how fast the centrifuge to obtain the right plasma citrate sample. As a result, plasma citrate can be in the condition of platelet-rich plasma (PRP) or platelet-poor plasma (PPP)[3].

All examination results cannot be separated from the error factor, these factors are instrumentation specifically coagulometer, centrifugation and comparison of blood with anticoagulants[4]. Accredited laboratories have carried out internal quality consolidation both pre-analytic, analytic and post-analytic to reduce errors in the measurement of clinical parameters. This research in an accredited laboratory wanted to find out whether there is a match between the two PPP and PRP methods in PPT and PPT and APTT examinations.

METHODS

This study is an experimental study aimed to test the suitability of the results between 2 blood preparation methods, namely the PPP and PRP samples in determining the value of APPT and APP. The research was conducted in a purposively chosen location, namely in an accredited clinical laboratory in the Kotabaru area of Yogyakarta City.

The research model is in the form of blood samples that are centrifuged with 2 different speeds, namely 3000 rpm for 10 minutes to get a platelet-poor sample (PPP) and 1000 rpm for 10 minutes to get a platelet-rich sample (PRP). The recommended container for holding plasma for hemostasis examination used silicon-coated glass tubes or from plastic materials, both of these materials have a

smoother inner surface so they do not spur platelet activation. Instrumentation including automatic pipettes, centrifuges, and coagulometers must always be routinely calibrated to ensure the quality of measurement results. Procedures to use the tools is carried out according to IKA (Tool Work Instructions). Laboratory asthe research site uses Sysmex CA-50 coagulometer.

Calculation of the minimum sample size used kappa calculations based on calculations by Lee (2000) for values of α = 0.05 and β = 0.1 (Power = 1-β). The assumptions of the free parameter values are p11 = 0.5, p.1 = 0.505, p1 = 0.505 and the assumption Δk = 0.2. In table 3.1, in Lee (2000), a minimum sample number (n) of 9 samples was obtained. If β is taken, 0.2 is obtained n = 7 and β = 0.05 is obtained n = 11. For this study, 10 samples were taken to meet the value of β = 0.1. The sampling technique used a purposive sampling quota method. The treatment using the PRP and PPP preparation methods used 10 paired samples, and then all received PPT and APPT were checked.[4]

Descriptive analysis was done by presenting the results in the form of tables and graphs to determine the gap or the difference between the high and low values of APTT, PPT for each type of plasma PRP and PPP. Statistical analysis was performed using the Kappa test to determine the suitability of the results of both methods using the Kappa Coefficient formula from Cohen (□) defined by Fleiss as:

$$\kappa = \frac{p_0 - p_e}{1 - p_e}$$

With $p_0 = p_{11} + p_{22}$ and

$p_e = p_{1.}.p_{.1} + p_{2.}.p_{.2}.$,

the calculation used 2 x 2 table of Kappa Coefficient.[4]

RESULTS AND DISCUSSION

Examination of differences in the value of Activated Partial Thromboplastin Time (APTT) and Plasma Prothrombin Time (PPT) in citrate plasma samples with Platelet Rich Plasma (PRP) and in Platelet Poor Plasma (PPP) conditions in clinical laboratories are accredited in the Kotabaru area of Yogyakarta for the remaining 10 remnants Patient's blood sample which is then treated with two centrifuges to obtain PPP and PRP to check the PPT and APTT values.A descriptive analysis was carried out to determine the maximum, minimum, average, and standard deviation value that can be seen in Table 2 below:

Descriptive analysis

Table 2.Descriptive analysis

Test	Preparataion	Minimum(second)	Maximum(second)	Average (Second)	Stadandard deviation	Score
PPT	PRP	11,0	12,2	11,6	0,40552	Normal
	PPP	10,3	11,9	10,97	0,49227	Normal
APTT	PRP	31,1	37,2	34,27	1,87679	Normal
	PPP	30,5	35,2	33,18	1.46652	Normal

To find out the results of the difference in the length of PTT values in plasma PPP and PRP are:

$$\frac{\text{Average value of PTTon PRP} - \text{Average value of PPT PRP}}{\text{Average value of PTT PRP}} \times 100\%$$

$$= \frac{11,6 - 10,97}{10,97} \times 100\%$$

$= 5,74$

The difference in PPT value in the PRP sample toward PPP is + 5.74 seconds, a value that is quite long and has the potential to become an abnormal value because the PPT reference value is 10.0-14.0 seconds with the difference in the value of the control plasma 2 seconds.

To find out the difference in results or the difference in the length of the APTT values in PPP and PRP plasma are:

$$\frac{\text{average value of APTTon PRP} - \text{average value of APPT PRP}}{\text{Average value of APTT PRP}} \times 100\%$$

$$= \frac{34,27 - 33,18}{33,18} \times 100\%$$

$= 3,28$

The difference in the APPT value in the PRP sample on PPP is + 3.28 seconds, a value that is relatively within the tolerance range, because the APPT reference value is at 25.0-35.0 seconds with the difference in control plasma is 5 seconds.

Kappa Analysis: Determination of Kappa test was to determine the suitability of the results of the two preparation methods (PPP and PRP). With Cohen's equation, it was obtained kappa value 1 for PPT and APTT examinations which showed a very good interpretation of the results of the compatibility between the two methods. Thus, the PPP and PRP preparation methods for examining APTT and PPT have a very high suitability of results.

From the results of the study, it was found that the tendency of platelet-rich plasma content (PRP) and platelet-poor plasma (PPP) is still within normal limits. APTT examination results in the PRP and the PPP sample statistically showed no significant differences. In the interpretation of PPT and APTT examination results, there

is a reference to state the pathology of the results of the test by referring to the value in the control plasma. For APTT, the tolerance of the test sample value to control plasma is + 5 seconds while in PPT the tolerance of the sample value with control plasma is 2 seconds. This means that if the test sample results are still within the tolerance range, the normal value criteria will be included.

The results of the study with the average of the samples on PPT values (normally 10-14 seconds) with tolerance value of PPT plasma control + 2 seconds obtained all results are in normal values. Whereas, The results of the APTT examination (normal value: 25-35 seconds) with tolerance to the APPT plasma control value of + 5 seconds, all samples were included in the range of normal values.

The PPP and PRP samples were technically different in how to obtain them, but the results of PPT and APTT examinations showed normal values. This is in accordance with the Kappa test with a value of 1 for PPT and APTT examinations, which means that the interpretation results are very good from the suitability of the two methods in the sample between PPP and PRP.

In accredited laboratories, there are already work standards for the preparation of APTT and PPT examination samples in the case of the centrifuge. In the laboratory where this study is located, the day-to-day concentration of citrate blood is done at a speed of 3000 rpm for 10 minutes (PPP plasma). The results of this study by comparing the centrifugation between 1000 rpm and 3000 rpm turns out that the results of PPT and APTT are still in the normal range, meaning that there is compatibility.

Looking at the difference in PPT results between PPP and PRP samples (5.74), it was shown that PRP plasma actually provides a longer value, whereas theoretically the more platelets will stimulate mechanical and chemical activity so that it will speed up the coagulation period.

Platelet cells in a small amount of plasma give a description when determining PPT and APTT that only the measured factors are extrinsic, intrinsic and joint pathways. This is because platelets contain significant amounts of various coagulation factors namely fibrinogen, factor V, von Willebrand factor, factor XI, factor XIII and High Molecular Weight Kininogen (HMWK). Some of these factors (fibrinogen, factor V, vWF, and HMWK) can trigger activation of clotting factors to increase coagulation activity, so plasma for PPT and APTT tests is recommended with platelet-poor samples.[5]

The content of fibrinogen in platelet cells is biochemically different from plasma fibrinogen. Surface-bound fibrinogen which is important for platelet aggregation is followed by induction by ADP and may be involved in other platelet functions. Von Willebrand Factor (vWF), a subunit of factor VIII which has a large molecular weight, is present in megakaryocytes, in platelet membranes, and in greater concentrations of α-granules. The plasma arrangement and platelet form of vWF bind to glycoproteins and glycolipids on platelet membranes, although only plasma vWF is important for normal platelet adhesion[5].

With the results of this study, according to researchers, it is necessary to analyze pre-analytic factors such as blood sampling, homogenization of samples, room temperature and instrumentation. Intake of blood with multiple stabbings, too long tourniquet placement allows blood samples to be mixed with thromboplastin tissue which will stimulate the extrinsic pathway (PPT) activity. Next, the sample that is not perfectly homogeneous with anticoagulants will start coagulation more quickly, as well as high ambient temperatures will increase the activity of clotting factors. Instrumentation factors, especially centrifuges and coagulometers which are not regularly calibrated can give inaccurate results.

All examination results cannot be separated from the error factor. The actual parameter values that will be determined from an analytic calculation are ideal sizes. This value can only be obtained if all causes of measurement errors are eliminated and the population is unlimited. Factors causing this error can be caused by various things including equipment, reagents, and measurement conditions and others. One way that can be used to reduce errors in analytical measurements is the calibration process[7].

Referring to the theoretical concept, then comparing with the results obtained, there are limitations to this study, especially the limited number of samples so that further research is needed with a higher proportion of samples to ensure that the sample for PPT and APTT examination is confirmed by PPP. Therefore, it is only an extrinsic factor of (PPT) and intrinsic (APTT) is corrected.

CONCLUSION

There is a very good compatibility between the PPP and PRP methods for PPT and APTT examinations (Kappa = 1).

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