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Effect of Variation Natrium Sitrat Concentration and Centrifugation Time on Prothrombin Time (PT) Value

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ABSTRACT

Background & Objective: Prothrombin Time (PT) test is one of the hemostasis tests used to determine whether there is a deficiency of extrinsic clotting factors and co-pathway clotting factors, namely factors I, II, V, VII, and X. The pre-analytical stage that must be considered in the hemostasis examination is the concentration of anticoagulant used and the centrifugation process. This study was conducted to determine the effect of variations in sodium citrate anticoagulant concentration and centrifugation time on Prothrombin Time (PT) values.
Method: The anticoagulants used in this study were 3.2% and 3.8% sodium citrate. Centrifugation is the process of separating cells from plasma. The effect of improper centrifugation is to increase the number of platelets, so that platelet factors contained in platelets will accelerate the formation of fibrin. The research conducted is a pseudo-experimental study by giving treatment to blood specimens that will be added with anticoagulant Sodium Citrate concentrations of 3.2% and 3.8% with a blood ratio of 9:1, then centrifuged at 3000 rpm and given a variation of time for 15 minutes, 10 minutes, and 5 minutes. The results were tested using Saphiro Wilk and Friedman Test.
Result : Based on the research that has been done, the Asymp. Sig. value obtained in the Friedman Test is >0.05. This indicates that the treatment that has been given to the sample has no effect on the value of Prothrombin Time.
Conclusion: It can be concluded that there is no significant difference between the variation of sodium citrate concentrations of 3.2% and 3.8%, as well as in the variation of 3000 rpm centrifugation time of 15 minutes, 10 minutes, and 5 minutes on the value of Prothrombin Time (PT).

Keywords: Prothrombin Time (PT); Anticoagulant Concentration; Centrifugation Time.

Introduction

The laboratory is a health facility that has the task of implementing in terms of measurement, determination and testing of materials of human origin. Most laboratory examinations are currently carried out using automated equipment, which greatly helps laboratory examinations to obtain accurate results (Fav Alvaro E.J. 2012).

Errors that lead to inaccurate results occur at the pre-analytical stage, analytical stage, and post-analytical stage. Among them, the biggest errors are in the pre-analytical stage, including hemolysis, insufficient specimen volume, illegible writing, wrong specimen, clots in the specimen, the amount of anticoagulant in the vacuum container, and the centrifugation process (Syauqiah NR, 2018).

Laboratory examinations consist of various types including hematological examinations to determine the overall condition of the patient and are often used for health checks (Ronald, A. Sacher, 2012). Hematology examination includes routine examination and special examination, special examination includes examination of hemostasis faeces. And one of the hemostasis function examinations is the Prothrombin Time (PT) examination (Zulaicha, 2010).

Prothrombin Time (PT) is one of the examination panels used to determine whether or not there is a deficiency in the activity of extrinsic and shared pathway clotting factors, namely factors I, II, V, VII and X. An elongated clotting time indicates a deficiency in one of these factors or in patients with oral anticoagulant therapy (Anisa, 2019).

The examination requires special attention, where pre-analytics play an important role that can affect the overall test results. The use of anticoagulants for hemostasis examination is anticoagulants containing citrate, generally sodium citrate is used.

In the laboratory, the types of sodium citrate concentrations that are often made are 3.2% sodium citrate and 3.8% sodium citrate. Sodium Citrate with a concentration of 3.2% is a type of anticoagulant recommended by the International Committee for Standardization in Hematology (ICSH) and the International Society for Thrombosis and Hematology for coagulation tests (Kiswari, 2014). Meanwhile, 3.8% sodium citrate was used to check the erythrocyte sedimentation rate. Administering anticoagulants with inappropriate concentrations will give inappropriate results (Mulyono, 2011).
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Some Hematology laboratories, which perform coagulation and ESR tests, only use one concentration of anticoagulant to streamline the materials used and save laboratory expenses.

In addition to anticoagulants, there are other factors that can affect the examination, namely specimen centrifugation time. In a study conducted by A. Sutan (2010), there was no significant difference in the examination (PT and aPTT) of the average number of platelets in citrated plasma centrifuged for 5 minutes at 3000 rpm and 10 minutes at 2000 rpm.

Some laboratories have no uniformity, especially citrate blood screening to obtain citrate plasma with little platelet content (Supartuti and Hardisari R, 2016). One of the reasons is because there are many references regarding centrifugation time, and a short time is needed to be able to immediately carry out the examination.

The concentration of sodium citrate anticoagulant and the length of centrifugation time can affect the results of the Prothrombin Time (PT) test. Inappropriate coagulant concentration may interfere with the effective binding of calcium ions, which in turn may affect the recorded clotting time. Inadequate length of

centrifugation time may lead to non-separation of red blood cell components which may affect the Prothrombin Time (PT) test results.

Objective

To determine whether there is an effect of varying concentrations of 3.2% and 3.8% Sodium Citrate anticoagulant with 3000 rpm centrifugation for 15 minutes, 10 minutes, and 5 minutes on Prothrombin Time (PT) values.

Method

The research method applied in this study is a quasi-experiment with a Statistics Group Comparison research design where there are two groups as the object of research. The first group received treatment while the second group did not receive treatment.

This study treated variations in the concentration of anticoagulant Sodium Citrate with concentrations of 3.2% and 3.8%, then performed 3000 rpm centrifugation with variations in time of 15 minutes, 10 minutes, and 5 minutes on the Prothrombin Time (PT) value.

The population and samples in this study were healthy individuals who did not have a history of disease and hemostasis disorders as many as 5 people.

The blood samples that have been obtained are then processed into plasma by examining the treatment in the form of adding variations in the concentration of sodium citrate anticoagulant and variations in centrifugation time.

The examination was carried out at the Hematology Laboratory of the Department of Medical Laboratory Technology of the Poltekkes Kemenkes Bandung from May to June 2024.

The type of data collected is primary data obtained from the results of the Prothrombin Time (PT) examination in plasma obtained by varying the concentration of sodium citrate anticoagulant and varying the centrifugation time. Measurement using the Quick One Stage method with the tools used are water bath, test tubes, and ose wire. Data processing in this study will be explained in tabular form and described narratively.

Results

The study was conducted in May 2024, by taking venous blood specimens from 5 healthy individuals without a history of disease and hemostasis abnormalities, and conducting Prothrombin Time (PT) examinations at the Hematology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Bandung. The examination was carried out by varying the concentration of anticoagulant Sodium Citrate concentration of 3.2% and 3.8%, then centrifuging 3000 rpm with a variation of time 15 minutes, 10 minutes, and 5 minutes. The normal value of Prothrombin Time (PT) is 11-18 seconds.

TABLE 1. Prothrombin Time (PT) Test Value

Nilai *Prothrombin Time* (Detik)						
Spesimen	Na Sitrat 3,2%			Na Sitrat 3,8%		
	15 Menit	10 Menit	5 Menit	15 Menit	10 Menit	5 Menit
1	15	14	14	14	15	14
	14	13	14	15	14	14
2	13	14	13	14	13	13
	13	14	14	13	13	13
3	16	15	15	16	14	15
	15	14	15	16	15	16
4	15	14	15	15	14	15
	15	14	14	14	15	15
5	13	12	12	12	13	12
	12	12	13	13	13	12
Rata-Rata	14,1	13,6	13,9	14,2	13,9	13,9

Based on Table 1, the average value of Prothrombin Time (PT) in the 3.2% Sodium Citrate variation with centrifugation for 15 minutes was 14.1 seconds. In the variation of 3.2% Sodium Citrate with centrifugation for 10 minutes is 13.6 seconds. In the variation of 3.2% Sodium Citrate with centrifugation for 5 minutes, 13.9 seconds. In the variation of 3.8% Sodium Citrate with centrifugation for

15 minutes, 14.2 seconds. In the variation of 3.8% Sodium Citrate with centrifugation for 10 minutes, 13.9 seconds. In the 3.8% Sodium Citrate variation with centrifugation for 5 minutes, 13.9 seconds. All Prothrombin Time (PT) values fell within the normal range of 11-18 seconds (TEClot PT-S, 2014).

The data were then statistically processed using the SPSS application with the Friedman Test.

TABLE 2. Friedman Test Statistic

	Variabel	Asymp. Sig.	Hasil	Kesimpulan
Antikoagulan Natrium Sitrat	3,2%	0,150	P >0,05	Tidak Terdapat Pengaruh
	3,8%	0,507	P >0,05	Tidak Terdapat Pengaruh
Waktu Sentrifugasi	15 Menit	0,705	P >0,05	Tidak Terdapat Pengaruh
	10 Menit	0,317	P >0,05	Tidak Terdapat Pengaruh
	5 Menit	1,000	P >0,05	Tidak Terdapat Pengaruh

Based on Table 2, the Asymp. Sig. > 0.05, so it can be It can be concluded that the variation of sodium citrate anticoagulant concentrations of 3.2% and 3.8% with 3000 rpm centrifugation varied by 15 minutes, 10 minutes, and 5 minutes does not have a significant effect on Prothrombin Time (PT) values.

Discussion

The principle of Prothrombin Time (PT) examination is to measure the time of plasma clot formation incubated at 37ºC. Then tissue thromboplastin reagent containing calcium ions (CaCl 0.22%) in the form of calcium chloride is added. The anticoagulant concentration used in the Prothrombin Time (PT) examination is the concentration of sodium citrate anticoagulant. 3.2% with a ratio of 9 parts of blood and 1 part of anticoagulant. Citrate serves to bind calcium ions (Ca2+) so that there will be no clotting process after the addition of anticoagulants. Before conducting the Prothrombin Time (PT) examination, specimens containing anticoagulants are centrifuged first to obtain citrate plasma. The time required according to the Clinical and Laboratory Standard Institute (CLSI) to obtain PPP by centrifugation at 3500 rpm for 10 minutes or at a lower speed for 10-30 minutes (Hardisari, R, 2016).

The use of 3.8% sodium citrate anticoagulant in the Prothrombin Time (PT) examination does not affect the results of the examination because sodium citrate functions in a specific and controlled manner in the process of collecting blood specimens. The sodium citrate used in this study is made from tri-sodium citrate powder dissolved in distilled water. The difference in concentration comes from the number of grams of powder used. Sodium citrate works by binding to calcium ions (Ca^{2+}) in the blood, which are necessary for the coagulation process. By binding calcium, Sodium Citrate prevents blood clotting during specimen collection and handling prior to analysis. At the time of examination, calcium is added back into the drawn plasma to restart the coagulation process (Setiabudy, 2009).

During the Prothrombin Time (PT) test, a reagent containing calcium chloride (CaCl2 0.22%) and thromboplastin to the anticoagulated plasma. Thromboplastin (tissue factor) added to plasma binds with calcium (Ca^{2+}) and activates the factor VII present in the plasma, becomes factor VIIa (activated), and continues until a stable fibrin network or clot is formed stable fibrin network or clot. This neutralizes the anticoagulant effect of sodium citrate and initiates the coagulation process measured as prothrombin time. As all specimens are treated in the same way, consistency and accuracy of results are maintained.

Before performing the Prothrombin Time (PT) test, plasma must be separated first because plasma contains the coagulation factors necessary to measure blood clotting time without interference from other blood

components. Plasma is the liquid part of blood that contains various proteins, including clotting factors.

Erythrocytes can cause hemolysis, which releases hemoglobin and other materials that can interfere with Prothrocyte measurements. Other materials that can interfere with Prothrombin Time (PT) measurements. Leukocytes can affect the results by releasing enzymes and chemicals unrelated to coagulation. Chemicals that are not related to coagulation. Therefore, plasma should be separated from other blood cell components to avoid interfering with the test (Puspitasari, 2017).

The centrifugation time does not affect the Prothrombin Time (PT) test because the purpose of centrifugation in this process is to separate plasma from blood elements blood elements. As long as the centrifugation is performed at a speed and time enough time to effectively separate the plasma, the resulting plasma quality will be adequate for Prothrombin Time (PT) analysis (Nugraha, 2015).

The coagulation factors in blood plasma measured in Prothrombin Time (PT) are fairly stable during routine centrifugation and handling processes. Thus, small variations in centrifugation duration will not affect the concentration or activity of these coagulation factors. Sodium Citrate concentration and centrifugation time do not have a significant effect on Prothrombin Time (PT) values when the test is performed with a volume of specimen, anticoagulant, and reagents according to the procedure.

Conclusion

Based on the results of the research that has been done, there is no significant effect of the Prothrombin Time (PT) value after getting treatment in the form of variations in anticoagulant concentrations of 3.2% and 3.8% with a centrifugation time of 3000 rpm for 15 minutes, 10 minutes, and 5 minutes.

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Conflict of Interest

There is no conflict of interest in the preparation of this research and article. This research was conducted without special funding support from any party.

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