CENTRIFUGE TIME VARIATIONS ON RECALCIFICATION PERIOD VALUE

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ABSTRACT

Hemostasis is the body's mechanism to stop bleeding during an injury. The recalcification period examination is a specific hematological examination of blood clotting disorders to look for deficiency of clotting factors from the intrinsic pathway (Factors V, VIII, IX, X, XI, and XII), prothrombin and fibrinogen. According to the standard operating procedure (SOP), the examination of the recalcification period of the citrate blood centrifuge is 20 minutes at a speed of 3000 rpm. Based on the facts in the field, samples were taken simultaneously, and several different examinations were carried out so that when the centrifuge was carried out, it was not by the SOP. So that there can be an error in the production of the examined citrate plasma. The study aimed to determine the effect of centrifuge time variation on the value of the recalcification period. The research method uses a quantitative approach, experimental research, and a cross-sectional research design. The sampling technique is Accidental Sampling, using nine samples from 9 patients with 3 treatments. The results of the data normality test with the Shapiro-Wilk test from the test results carried out can be seen in 3 data groups; from the data obtained, there is one abnormal treatment. So, the Kruskal-Wallis Test was continued, and the Kruskal-Wallis test was non-parametric. The conclusion obtained from the study is that there is an influence on the recalcification period.

Keywords: Centrifuge Time, Recalcification Period

INTRODUCTION

Blood transports oxygen, carbon dioxide, nutrients and metabolites throughout the body. Blood also has a role as a tool for acid-base balance, protecting against infection, and maintaining body temperature. Blood is composed of 2 components, namely plasma and blood cells. Blood cells are composed of red blood cells, leukocytes, and platelets [16].

Hemostasis is part of the mechanism that causes bleeding to stop from *blood vessels* . This is a process that includes a number of interrelated steps. The stages of the hemostasis mechanism are classified into four stages [16] namely narrowing of *the blood vessels* , formation of a temporary "platelet plug", activation of the coagulation cascade, formation of a "fibrin plug or final clot. Hemostasis is the body's normal mechanism for stopping bleeding in an injured area. Hemostasis is *a response* to stop blood loss carried out by *blood vessel spasm* , adhesions, platelet aggregation, and the active participation of coagulation factors. Hemostasis includes modulation of vascular endothelium, platelet aggregation, and activation of various coagulation pathways remains in the *blood vessel system* . The main function of the clotting mechanism is to maintain *blood clotting* so that blood can flow properly in the circulatory system [16].

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Excessive bleeding will prolong the operation time and increase the risk of surgery and even lead to medical malpractice. Effective control of blood loss can save time and increase patient survival rates [11]. When bleeding occurs, hemostasis is the body's spontaneous response. The mechanism of hemostasis *in vivo* includes two processes: primary hemostasis, when the endothelium is injured, and collagen and other subendothelial matrix components are exposed, and *von Willebrand factor* is released to allow platelets to adhere to the wound site; secondary hemostasis, tissue factor stimulates the conversion of prothrombin to thrombin, and soluble fibrinogen acts to limit the formation of insoluble fibrin clots [10]. However, if bleeding occurs, the body does not rely on the natural hemostasis process to control blood loss, and an effective external hemostasis method is needed to control blood loss in the body. Therefore, additional methods are needed to make bleeding stop quickly along with medical assistance [26].

blood clotting process begins when platelets and other plasma factors come into contact with an abnormal surface, such as a damaged *blood vessel* . When a wound occurs on the surface of the body, platelets, which are blood components, gather in the area where the wound is and clog and cover the wound. The recalcification period examination is a special hematological examination as a screening examination for abnormal *blood clotting to look for various clotting factors that are deficient in the intrinsic pathway (Factors V, VIII, IX, X, XI, and XII), prothrombin and fibrinogen.* This examination measures the time required to prepare fibrin from low-platelet plasma which does not contain $Ca2^{+}$ with CaCl2 added. To achieve low-platelet plasma, centrifuge or *centrifuge is carried out* at a speed of 3000 rpm for 20 minutes so that the plasma only contains a few platelets [9] .

Referring to the fact that in the field samples are taken simultaneously, new specimens are sent to the laboratory after blood from all patients has been taken and a number of different tests have been carried out so that when *the centrifuge* is carried out it usually does not comply with standard operating procedures (SOP). So there could be errors in producing the citrated plasma that will be examined. To obtain a good citrate plasma sample according to standard operating procedures (SOP), the citrate blood *centrifuge recalcification period* is 20 minutes at a speed of 3000 rpm. However, to shorten the *centrifuge* inspection process , here the researcher wants to carry out an experiment with *the centrifuge time* at a speed of 3000 rpm for 15 minutes and also try it for a longer time, namely approximately 25 minutes at the same speed of 3000 rpm.

Calibration is a series of activities that establish the relationship of the values displayed by a measuring instrument or measurement system, or represented by measuring materials, to relevant and known measuring values in a particular situation. Calibration is the activity of establishing the conventional truth about the value of an instrument or measurement material by comparing it with a measurement standard for a fluid sample taken from the patient's body. The advantage of calibration is that it maintains the condition of the measuring instrument and the material being measured according to specifications. This supports the quality systems used on testing equipment in various hospitals and allows them to determine the difference (deviation) in the correct price and the price displayed by the meter [12].

RESEARCH METHODOLOGY

The approach used in the research, namely a quantitative approach, was used to determine the effect of *centrifuge time* on the recalcification period. The type of research used is experimental research, with a *cross-sectional research design* which aims to determine the effect of *centrifuge time* on the recalcification period. The location of the

research was carried out at the Bina Mandiri University Gorontalo instrument laboratory and the research was carried out in July 2024.

The research used a population of each active student at Bina Mandiri University, Gorontalo. The sample size used 9 samples taken from 9 patients with 3 treatments, so the total number of research subjects was 27 examinations that would be carried out during the recalcification period, for the sampling technique using *Accidental sampling* of active students at Bina Mandiri University, Gorontalo.

2.1 Tools and materials

Tools used in research include *tourniquets , stopwatches* , test tubes, *centrifuges , water baths,* analytical balances, *spatulas,* micropipettes, dropper pipettes, tips (yellow and blue). Materials used in the research include 70% alcohol, venous blood samples, patient plasma, *distilled water,* 3.2% sodium citrate anticoagulant, 0.025 M CaCl2, and 0.85% NaCl and alcohol swab or (alcohol swab)

2.2 Work Procedures

Venous Blood Collection

When taking venous blood, respondents were asked to sit next to a table as a support. Place your arms on the table and place pads under your elbows. The choice of vein is chosen by the hand that carries out a lot of activity, see whether the vein is visible or can be felt, if visible, choose the median *cubital* or *cephalic vein* . Place *a tourniquet* approximately 10 cm above the elbow, then disinfect the area to be pierced using 70% alcohol. Visible vein puncture, 3 mL of venous blood is taken. When the blood is aligned to where it needs to be, release the *tourniquet* . Place dry cotton over the needle then remove the needle when the volume we need is met, then stick a plaster on the puncture site.

Preparation of Plasma Sodium Citrate

A total of 0.1 ml of 3.2% Na Citrate solution was put into a tube and 0.9 ml of venous blood was added and homogenized. *centrifuge* at a speed of 3,000 rpm for 10 minutes, 15 minutes, 20 minutes. After that, the plasma that has been formed is separated using a micropipette.

Recalcification Period Examination Procedure

1. Before the examination, incubate the CaCl2 solution, 0.85% NaCl, and the patient's plasma (plasma citrate) in a 37℃ water bath until the fluid temperature reaches 37℃.
2. Place the tube in a water bath at 37℃.
3. Put 0.1 mL of plasma and 0.1 mL of 0.85% NaCl solution into the tube, then incubate at 37℃ for 1-2 minutes.
4. Add 100 µL of CaCl2 solution to the tube containing plasma and NaCl, stir, and start the stopwatch.
5. Let the mixture sit for 90 seconds, then remove the tube from the water bath and check for clot formation.
6. Stop the stopwatch when a clot forms and record the time as the recalcification period.

Interpretation of Results ; The normal value of the recalcification period is 90-250 seconds

RESEARCH RESULT

3.1 Univariate Analysis

T able 1 Research Result Data

No.	Recalcification Period (Seconds)		
	15 minutes	20 minutes (K)	25 Minutes
1.	128	142	224
2.	124	135	179
3.	133	360	540
4.	199	235	273
5.	190	200	220
6.	180	215	218
7.	120	225	242
8.	160	212	230
9.	210	214	285

Source : Primary Research Data, 2024 .

Table 3.1 Data from research results examining the recalcification period with three treatment groups, achieved normal

results (90-250 seconds) in plasma in *the centrifuge* for 15 minutes, all achieved normal results. Meanwhile, when the plasma was treated in *a centrifuge* for 20 minutes, there were plasma samples with abnormal results (>250 seconds), 1 plasma sample in sample code III with an abnormal result of 360 seconds. Then in the final treatment where the plasma samples were *centrifuged* for 25 minutes there were 3 plasma samples with abnormal results (>250 seconds) in codes III, IV and IX with abnormal results of 540 seconds, 273 seconds and 285 seconds respectively.

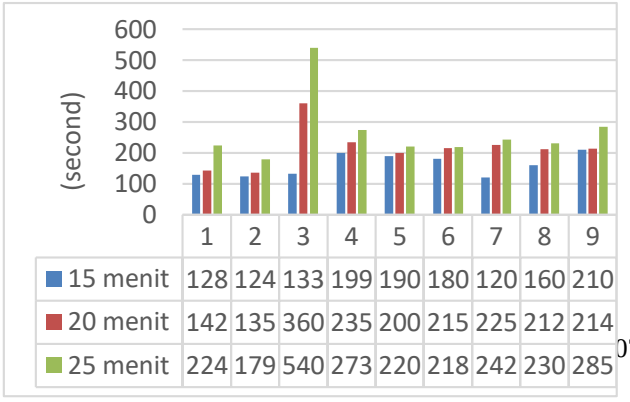
T able 2. Description of examination results for the recalcification period with long *centrifuge treatment* (15 minutes, 20 minutes and 25 minutes).

Centrifuge Time	Recalcification Period		
	Lowest	Highest	Average
15 minutes	120	210	160.4
20 Minutes (Control)	135	360	215.3
25 Minutes	179	540	267.8

Source : Primary Research Data, 2024 .

The results achieved from the Recalcification Period examination with three treatment groups will be clearly displayed in a bar diagram, namely.

Figure 1. Bar diagram of examination of the recalcification period in three long treatment groups. *Centrifuge* bar diagram of blood glucose examination results



Source : Primary Research Data, 2024 .

Referring to Table 2 and Figure 1, the bar diagram of the results of the research description shows that of the 9 respondents studied, the results of the examination of the recalcification period were the lowest when the plasma samples were treated in *the centrifuge* for 15 minutes with a result of 120 seconds, while the plasma samples were treated in *the centrifuge* for 20 minutes with a result of 120 seconds. results in 135 seconds, and when treating plasma samples in *the centrifuge* for 25 minutes with results in 179 seconds. Meanwhile, the highest value results were in examining the recalcification period by treating the plasma sample in *the centrifuge* for 15 minutes with a result of 210 seconds, when treating the plasma sample in *the centrifuge* for 20 minutes with a result of 360 seconds and treating the plasma sample in *the centrifuge* for 25 minutes with a result of 540 seconds. The average value of the recalcification period examination with three plasma treatments was achieved respectively in *the centrifuge* for 15, 20 and 25 minutes with results (160.4 seconds, 215.3 seconds and 267.8 seconds).

3.2 Bivariate Analysis

Table 3 . *Shapiro-Wilk* Normality Test Results

Results	Statistics	*Sig* value
Recalcification Period	,777	**,000**

Source : Primary Research Data, 2024 .

Referring to table 3. above, the results of the data normality test using the *Shapiro-Wilk test* from the results of the tests that have been carried out can be seen in the data,

namely citrated blood which was *centrifuged* for 15 minutes, 20 minutes and 25 minutes. There is data that has a p value below 0.05, so it can be confirmed that the data is not normally distributed. Therefore, it can be emphasized that the data in this study is not normally distributed and is continued with non-parametric comparative analysis testing, non-parametric comparative tests using the *Kruskal-Wallis test* .

Table 4. *Kruskal-wallis Test Results*

Kruskal-wallis test	*Sig.* (2 *-tailed*)
Chi-square	12,940
Df	2
Asym. Sig	**,001**

Source : Primary Research Data, 2024 .

Table 4 shows the results of the *Kruskal-Wallis* test , where 9 respondents achieved a test result of 0.001, where the basis for decision making or interpretation of the results of the *Kruskal-Wallis test* is that if *the Asymsig value* or significance value is above 0.05 then there is no influence on *the dependent. variable* and *independent variable* , and if the value is below 0.05 then there is an influence on *the dependent variable* and *independent variable* . Therefore, it can be concluded from the results of this study that there is an influence of variations in *centrifuge time* (15 minutes long, 20 minutes long and 25 minutes long) on the results of the recalcification period examination as indicated by the results of the research hypothesis, namely 0.001 (< 0.05).

Table 5. *Kruskal-wallis analysis calculation results*

Centrifuge Time	H Count	H Table	Conclusion
Three centrifuge time treatments	12,940	5,990	H0 is rejected

Source : Primary Research Data, 2024 .

In table 5. Calculation Results of the *Kruskal Wallis* Test Analysis , the df value is the degrees of freedom where there are 3 treatments minus 1 and the df value is 2. The calculated H value obtained in the *Kruskal Wallis test statistic is* 12,940 and the H table is obtained in the *chi square table* in degrees of freedom with The confidence level of 0.05 or 5% was achieved at 5,991.

DISCUSSION

Hemostasis examination in bleeding and thrombotic disorders is very important because it can provide important information for diagnosis, prognosis and monitoring therapy. Recalcification time examination is a blood test to detect deficiencies in intrinsic pathway coagulation factors (factors V, VIII, IX, X, XI, XII), prothrombin and fibrinogen. Special hematology tests used to screen for coagulation disorders. In this study, we measured the time required to produce fibrin from Ca2+-free platelet-poor plasma upon addition of CaCl2. To obtain plasma with a low platelet content, run the centrifuge or centrifuge for 20 minutes at a speed of 3000 rpm so that the platelets only contain a portion of the platelets [11].

The study of recalcification time was influenced by platelets. The more platelets in the plasma, the shorter the recalcification time.

To get rid of platelets, what is recommended is to run *a centrifuge* with a speed of 3000 rpm for 20 minutes so that the plasma contains few platelets. Hemostasis examination is greatly influenced by preanalytic factors in plasma citrate production. Therefore, you need to consider a number of factors, such as: (blood collection method, dosing and mixing citrated blood, centrifugation, transportation and storage of samples). Elements of technological analysis of tool inspection or tool calibration equipment. According to *the Clinical and Laboratory Standard Institute* (CLSI), PPP is achieved by centrifugation at 2000 g (3000 rpm) and low speed for 10 to 30 minutes. not suitable because it takes too long or too fast in the *centrifuge process* so that an inappropriate citrate plasma sample is obtained because there is no uniformity in the length and speed used by each laboratory. As a result, citrated plasma is in the

condition of platelet-rich plasma (RPR) or platelet-poor plasma (PPP) [12].

A centrifuge is a device that spins a sample at a speed that causes heavier particles to collect at the bottom of the *centrifuge tube* . The speed *of the centrifuge* matters because the higher the speed, the faster the platelet deposition will occur and vice versa. Apart from speed, *centrifuge time* also affects test results. The longer the centrifuge time, the greater the results. If the *centrifuge time* is too short, the substances involved will not be completely separated **.** Meanwhile, if the *centrifuge time* is too long, it will damage the sample to the point of lysis (Aghniya, 2018). This condition is proven by a change in the results after *centrifuge* from normal results to abnormal results in sample codes III, IV, and IX. The results of the research data show that in sample code III the 15 minute *centrifuge time treatment showed a recalcification period of 133 seconds, then there was a change (increase) after the 20 minute centrifuge time treatment was carried out* to 360 seconds, and there was another change (increase) in the *centrifuge time treatment* for 25 minutes to 540 seconds. Sample code IV shows the recalcification period in the 15 minute *centrifuge time treatment. It shows the recalcification period is 199 seconds, then there is a change (increase) after the 20 minute centrifuge time treatment is carried out* to 235 seconds, and there is another change (increase) in the *centrifuge time treatment.* for 25 minutes to 273 seconds. Finally, sample code IX shows the recalcification period in the *centrifuge time treatment* of 15 minutes. It shows the recalcification period is 210 seconds, then there is a change (increase) after the 20 minute *centrifuge time treatment is carried out* to 214 seconds, and there is another change (increase) in the treatment time. centrifuge for 25 minutes to 285 seconds. Apart from abnormal results, there were also changes (increases) in normal results in all citrate plasma samples that were *centrifuged* . This data shows that with control using *a centrifuge time* of 20 minutes which is used as a reference procedure, the researchers concluded that *centrifuge treatment* at a time above the reference (20 minutes) can prolong the recalcification period and *centrifuge treatment* at a time below the reference (20 minutes) can shorten the recalcification period [14]

Examination of the recalcification period is influenced by platelets, while to get rid of platelets it is recommended to use low-platelet plasma which contains few platelets with centrifugation for 20 minutes at a speed of 3000 rpm. Under normal circumstances the recalcification time is between 90-250 seconds. In the *centrifuge treatment* > reference time (20 minutes), namely at a *centrifuge time* of 25 minutes, 3 samples were achieved that changed (increased) to become abnormal, which should have been normal samples seen in the control group. This increase in results occurred because the centrifuge time was too long resulting in citrated plasma in platelet-poor plasma (PPP) conditions with a prolonged rectification period. The opposite of the centrifuge treatment < reference time (20 minutes), namely at a centrifuge time of 15 minutes, all samples experienced changes (decreased) but all were still in normal condition as seen in the control group. This decrease in results occurs because *the centrifuge time is too fast* resulting in citrated plasma in platelet-rich plasma (PRP) conditions shortening the rectification period. So it can be concluded that the > reference time (20 minutes) in that condition (25 minutes) citrate plasma is in platelet-rich plasma conditions with a prolonged rectification period, while the < reference time (20 minutes) in that condition (15 minutes) citrate plasma is in plasma condition. poor platelets with a shortened rectalification period [11].

Description of Results and Research Bar Diagram obtained from the 9 respondents studied who had the lowest score on the results of the Recalcification Period

examination by treating the plasma sample in *the centrifuge* for 15 minutes with a result of 120 seconds, when treating the plasma sample in the *centrifuge* for 20 minutes with a result of 135 seconds, and in the treatment The plasma sample was *centrifuged* for 25 minutes with a yield of 179 seconds. Meanwhile, the highest value results were in examining the recalcification period by treating the plasma sample in *the centrifuge* for 15 minutes with a result of 210 seconds, when treating the plasma sample in *the centrifuge* for 20 minutes with a result of 360 seconds and treating the plasma sample in *the centrifuge* for 25 minutes with a result of 540 seconds. The average value of the recalcification period examination with three plasma treatments was obtained respectively in *the centrifuge* for 15 minutes, 20 minutes, and 25 minutes with results (160.4 seconds, 215.3 seconds, and 267.8 seconds). In this case there were very high examination results, namely in sample number 3 which achieved results that were far from the usual normal values, with the results achieved being 360-540, this condition could be said to be a symptom of hemophilia. Hemophilia is a genetic disease that disrupts the blood clotting process. The main symptom of hemophilia is prolonged bleeding. This disease occurs more frequently in men than in women [8].

Changes (increases) occurred in the average value of the recalcification period from the *centrifuge time* of 20 minutes and 25 minutes. In the recalcification period, the plasma was centrifuged for 25 minutes, giving abnormal results (> 250 seconds) with an average value (267.8 seconds). These results experienced strong changes which can be seen in the normal control results (90-250 seconds) with an average value (215.3 seconds). This change can certainly provide information on the sample centrifugation process. It is highly discouraged to carry out the centrifugation process for too long (>20 minutes) to achieve low platelet plasma for the recalcification period. The opposite result, namely (a decrease) occurred in the average value of the rectification period from a centrifuge time of 20 minutes to a time of 15 minutes. The results of the recalcification period of plasma treatment in *the centrifuge* for 15 minutes showed that the results were still in normal condition (90-250 seconds) with an average value (160.4 seconds). This result experienced a decrease in changes not too far seen from the normal control results (90-250 seconds) with an average value (215.3 seconds). This change provides information that the sample centrifugation process has not achieved the correct results as desired by the centrifugation process to achieve low platelet plasma for the rectalification period [21].

Similar results to Wiarsih S. The results of the research achieved the average recalcification period results in citrate plasma at centrifuging speeds of 2000 rpm, 3000 rpm and 4000 rpm respectively, namely 122.4 seconds, 185.5 seconds and 244.6 seconds. These results show that there is a change in the results of the rectification period at different centrifuging speeds. When using *a centrifuge*, the centrifuge time (time) and speed are things that must be considered to achieve appropriate results. Factors that influence the results of the recalcification period are antiagulants, temperature, storage time, citrate volume, and low platelet plasma. When examining the recalcification period, low platelet plasma is used. The greater the number of platelets, the shorter the recalcification (Gandasoebrata, 2007). So it is concluded that the *centrifuge process* is not recommended to be too long and not too fast. This condition is proven by the results of the control group (20 minutes long) and the experimental group (15 minutes long and 25 minutes long) which achieved an average value in the range of quite high differences marked by the average value (normal) becomes (abnormal) in the centrifuge treatment (for 20 minutes) [20].

Shapiro-Wilk normality test , referring to Santoso (2014), in the *Shapiro Wilk test* data

is said to be normally distributed if *the significant value* exceeds *the significance level* (5% or 0.05). Referring to the results of the normality test on the effect of variations in *centrifuge time* on the rectification period, the results of the experimental group, namely *the centrifuge* (15 minutes and 25 minutes), were (0.18 and 0.01) on the 25 minute *centrifuge* <0.05, so it is known that the data is not normally distributed. Apart from that, the results of normality testing in the control group, namely *the centrifuge* (for 20 minutes) were (0.06) > 0.05, the data was normally distributed. Referring to these results, it can be concluded that the data obtained is not normally distributed so that it can be continued with non-parametric comparative analysis testing, namely the *Kruskal Wallis test* .

Kruskal-Wallis Test Results , to determine the differences in the results of the rectification period with variations in *centrifuge time* (15 minutes, 20 minutes and 25 minutes), data analysis was carried out using *the Kruskal-Wallis Test.* The results *of the Kruskal-Wallis Test* in the inspection results of the rectification period achieved a value of *Asymp sig* = 0.001 (< 0.05), meaning that H0 was rejected while Ha was accepted, meaning that there was a difference in the variation of *centrifuge* time (15 minutes, 20 minutes and 25 minutes) compared to results of the rectification period.

CLOSING

Conclusion

The results *of the Kruskal-Wallis Test* on the results of the examination of the recalcification period achieved *an Asymp value of sig* = 0.001 (< 0.05), meaning that the null hypothesis (H0) was rejected while the alternative hypothesis (Ha) was accepted, meaning that there was a difference in the variation in *centrifuge time* (15 minutes long). , 20 minutes and 25 minutes) on the results of the recalcification period.

result = 12,940 and the chi square table is 5% for df (2) = 5.991 so it can be confirmed that the value of Ho is rejected and Ha is accepted, meaning that there is an influence on the variation of *centrifuge time* (15 minutes, 20 minutes and 25 minutes) on the mass results. recalcification.

Suggestion

1. For Medical Laboratory Technology personnel, the examination of the recalcification period when carrying out the centrifugation process should be carried out within 20 minutes, no less or more, to avoid errors in the examination results.
2. The next researcher is advised to continue this research by including speed variations in citrate plasma with time variations of 2500 rpm, 3000 rpm and 3500 rpm. To see whether there is an influence when using different *centrifuge* speeds in examining the recalcification period.

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