EFFECT OF VARIATIONS OF BLOOD CLOTTING TIME BEFORE CENTRIFUGATION ON CHOLESTEROL LEVELS WITH ENZIMATIC METHOD

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Abstract

Introduction: Pre-analytic is an important phase of laboratory examination. One of them is how to get serum. The stage of blood coagulation is important in obtaining a good serum. This phase is very influential on the quality of the serum. The reality in the field during clinical chemistry examinations is that there is a time difference in the clotting of blood samples to become serum. The purpose of this study was to determine the effect of variations in blood clotting time before centrifugation on cholesterol by the enzymatic method.

Method: The method used in this research is quantitative analysis with the type of experimental research. The treatment given to the object is the variation of blood clotting time before centriugation with a time of 10, 20 and 30 minutes.

Results: The results of the average measurement of cholesterol levels with blood clotting time of 10,20 and 30 minutes were 133.94 mg/dl, 148.23 mg/dl and 162.31 mg/dl. Cholesterol levels with clotting time of 10 and 30 minutes increased by 21% and clotting time of 20 and 30 minutes by 9%. The data obtained were statistically analyzed using the Oneway ANOVA test for clotting time 10 with 30, the results obtained sig 0.003 and coagulation time 20 with 30 obtained sig 0.214.

Conclusion: The results of this reserch indicate that between groups of 10 with 30 minutes of clotting time there is a significant effect on cholesterol levels and clotting time of 20 with 30 minutes has no significant effect on cholesterol levels.

Key words *: :* CHOD-PAP, cholesterol, clotting time, *oneway annova*, serum

INTRODUCTION

Lipid is water insoluble molecules such as cholesterol and triglycerides. Lipids transported by proteins in thebloodstream are called lipoproteins. Lipids are produced in the liver and obtained from foods such as meat and dairy products. Lipid and lipoprotein profiles are generally used as a normal assessment of elevated blood cholesterol levels (hypercholesterolemia) and elevated blood levels of fatty substances (hypertriglycerides) associated with an increased risk of cardiovascular disease (Fristiohady & Ruslin, 2020).

Measurement of cholesterol levels serves to determine the level of cholesterol in the blood. In the clinical chemistry measurement guidelines of the Ministry of Health Number 1792/MENKES/SK/XII/2010 regarding cholesterol assessment, the technique used is CHOD PAP (*Cholesterol Oxidase-Peroxidase Aminoantyphirin*). Cholesterol examination using serum or plasma specimens. Serum is a liquid blood containing clotting factors that does not use anticoagulants when collecting serum specimens. Complete coagulation takes 30 minutes, depending on the presence of clot activator. The sample was then centrifuged to separate blood cells and serum (Warekois & Richard Robinson, 2017). The main difference between plasma and serum is that serum does not contain and some potassium is released from platelets (serum potassium is slightly higher in serum than plasma (Bishop et al., 2013).

Pre-analytic is an important part of laboratory examination. One example is how to obtain serum. The stage of blood coagulation is important in obtaining a good serum. The blood clotting phase before centrifugation has an influence on the quality of the serum. Serum is obtained from blood that is accommodated in a tube without anticoagulant and allowed to clot for 30 minutes then centrifuged using 3000 RPM for 15 minutes and will produce a clear yellow liquid (Nugraha, 2015). When in the field the fact in clinical chemistry examinations, there are differences and variations in the time of blood clotting before centrifugation to become serum. There is a difference in blood clotting time from the time specified to shorten the time, this method is actually not in accordance with the procedure. The difference in blood clotting time before

centrifugation in the manufacture of serum that is not according to the procedure then the serum can cause differences in the interpretation of the results.

Research conducted by Adi (2019) revealed that there was a significant difference in cholesterol levels fromclot blood before centrifugation and direct centrifugation. Similarly, research conducted by Sari (2018) foundthat there was a significant difference in HDL cholesterol levels from blood that was directly centrifuged andclot before being centrifuged. On the basis of this background, the researchers wanted to conduct research onthe effect of blood clotting time on cholesterol levels. Based on this problem, researchers are interested in knowing the effect of blood clotting time before centrifugation on cholesterol levels by the enzymatic method. This study provides variations in blood clotting time 10 minutes, 20 minutes and 30 minutes before centrifugation. Whereas in the previous study only treated blood that was clot and not clot before centrifugation.

METHOD

This type of research is an experimental quantitative research. The study was conducted in February-June 2022 at Clinical Chemistry Laboratory, East Bekasi. The population in this study were laboratory workers at Private Hospital East Bekasi, who were willing to have blood drawn with informed concern. The samplingtechnique in this study was purposive sampling. There were 17 respondents. The inclusion criteria in this study were laboratory workers at Private Hospital, East Bekasi, who were <30 years old. Exclusion criteria in this study were icteric, lipemic and lytic serum.

A. Tools and Materials
   1. Tools
      The tols used in this study were Semi Chemistry Analyzer (Ratyo RT-1904C), micropipette (Dragon lab) (10 and 1000 μL), tube rack, tip, bath, centrifugation, tourniquet, holder.
   2. Materials
      The materials used in this study were cholesterol reagent (Dumolabs), distilled water, handscoon, mask, plain vacutainer, needle, swab alcohol, plaster, yellow and blue tips.

B. Procedure
   1. Pre Analytic
      The pre analytical stage in the study begins with blood taking done and preparing tools and materials. Next, the patient is identified and then a tourniquet at a distance of 3-4 cm from the puncture site and palpated to determine which vein to use. Venous blood sampling can use the median cubital vein, saphalic vein and basilic vein. Clean the puncture site with 70% alcohol and let it dry for 30-60 seconds. Then the vein is pierced with a needle and the blood will enter the plain vacutainer tube. Then the blood of each tube was labeled 10 minutes, 20 minutes and 30 minutes.
   2. Analytic
      The analytical step was carried out by making serum with clott times before centrifugation, namely 10 minutes, 20 minutes and 30 minutes. After obtaining the serum, cholesterol was checked. Examination of cholesterol in serum is done by preparing 3 test tubes labeled blank, reagents and samples. The examination of cholesterol levels in this study was carried out in duplicate. In the blank tube was added 10 μl of distilled water and 1000 μl of cholesterol reagent. In the standard tube 10 μl of standard reagent and 1000 μl of cholesterol reagent were added and 10 μl of serum and 1000 μl cholesterol reagent were added to the sample tube. Then the blank, standard and sample tubes were incubated at 37°C for 10 minutes using an incubator/waterbath. Then the cholesterol level was measured using a semi chemistry analyzer with a wavelength of 500 nm. The post-analytic stage was carried out by entering cholesterol levels into the SPSS version 26 program.

RESULTS

This research was conducted at the clinical chemistry laboratory, East Bekasi. Blood sampling was carried out by 17 respondents consisting of women and man. The number of women amounted to 16 people or 94%and man amounted to 1 person or 6%. With respondents who fit the inclusion and exclusion criteria. The volume of blood taken by each respondent was 9 ml of blood. Examination of cholesterol levels using serumusing blood clotting times of 10, 20 and 30 minutes. Based on the results of the examination of cholesterol levels of 17 respondents whose blood was given variations in clotting time, the cholesterol levels were obtained as follows:

Table 1. The result of the average level of cholesterol on the variation of blood clotting time

Times	N	Minimum (mg/dL)	Maksimum (mg/dL)	Mean (mg/dL)	Std.
10 minute	17	83,67	178,26	133,94	22,52
20 minute	17	89,22	189,20	148,23	27,23
30 minute	17	120,32	192,38	162,27	22,04

Based on table 1 shows that the lowest cholesterol level in the 10 minute clotting time was 83.67 mg/dL, thehighest was 178.26 mg/dL, and the average was 133.94 mg/dL. Cholesterol levels during the clotting time of20 minutes increased. The lowest was 89.22 mg/dL, the highest was 189.20 mg/dL, and the average was 148.23 mg/dL. At a clotting time of 30 minutes, the lowest level was 120.32 mg/dL, the highest was 192.38mg/dL, and the average was 162.27 mg/dL.

Table 2. The Statistical Test *Oneway Annova* between time groups

Clotting Time	Clotting Time	Sig.	Interpretation
30 minute	10 minute	0,003	There was a significant effect between clotting times of 10 and 30 minutes on cholesterol level
	20 menit	0,214	There was no significant effect between clotting times of 20 to 30 minutes on cholesterol levels.

Statistical test in table 2 by comparing the time groups, it was found that the clotting time of 10 to 30 minutes obtained a sig value of 0.003, which means that there is a significant effect between the clotting time of 10 minutes and 30 minutes on cholesterol levels, while at the blood clotting time of 20 to 30 minutes, the sig value is 0.214. which indicates that there is no significant effect between clotting times of 20 minutes and 30minutes on cholesterol levels. tes obtained a sig value of 0.003, which means that there is a significant effect between the clotting time of 10 minutes and 30 minutes on cholesterol levels, while at the blood clotting time of 20 to 30 minutes, the sig value is 0.214. which indicates that there is no significant effect between clotting times of 20 minutes and 30 minutes on cholesterol levels.

DISCUSSION

Cholesterol levels were tested using the colorimetric enzymatic method, namely CHOD-PAP. The principleof this method is that cholesterol in the presence of the enzyme cholesterol esterase will form fatty acids andfree cholesterol. The free cholesterol formed is then converted into cholesterol-4-en-3-on and *hydrogen peroxide* (H_2O_2) by the enzyme *cholesterol oxidase* (CHO). Hydrogen peroxide with phenol and 4- aminophenazone by the enzyme *peroxidase* (POD) will be converted into colored compounds. The

absorbance of this color is proportional to the cholesterol in the sample (Nugraha & Badrawi, 2018).

Based on table 1 , the percentage increase in blood cholesterol levels by comparing cholesterol levels with aclotting time of 10 to 20 minutes shows an increase in cholesterol levels by 10%. At the freezing time of 10 to 30 minutes, there was a significant increase of 21% and at the freezing time of 20 to 30 minutes, there wasan increase in cholesterol of 9%. This result is in accordance with the theory that the specimen has not undergone complete coagulation, which will cause the fat content to not be completely released so that it affects the fat content (Nugraha, 2015).

Research conducted by Adi (2019) showed that there was a significant difference in cholesterol levels from frozen blood before centrifugation and direct centrifugation. The pre-analytic stage has a significant impact on the analysis of fat content, such as checking cholesterol and triglyceride levels. Blood that was first centrifuged directly without a coagulation process will produce less serum with blood that has undergone a complete coagulation process. This is because the coagulation process is not perfect and the fat content is still bound in the serum.

Sari (2018) revealed that in examining HDL cholesterol levels there were differences in HDL levels betweenblood that was directly centrifuged and frozen before centrifugation. According to the Decree of the Ministerof Health of the Republic of Indonesia Number 1792/MENKES/SK/XII/2010 concerning guidelines for clinical chemistry examination in obtaining serum, the blood is allowed to coagulate for 30 minutes and centrifuged using a speed of 3000 RPM for 5-15 minutes. To avoid changes in dissolved substances due to hemolysis effects, serum examination is carried out in less than 2 hours.

CONCLUSION

Based on the *Oneway Annova* test between the 10 minute and 30 minute clotting time group sig 0.003 was obtained, it can be concluded that there is a significant effect between the 10 minute and 30 minute clotting time on cholesterol levels. At the clotting time of 20 minute and 30 minute, sig 0.214 was obtained, which indicated that there was no significant effect between the clotting time of 20 and 30 minute on cholesterol levels.

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