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Comparison of Total Protein Levels in Lipemic Serum with Alpha Cyclodextrin, Gamma Cyclodextrin, and High-Speed Centrifugation Treatment

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ABSTRACT

Lipemic serum can interfere with examination parameters that require light transmission and may lead to inaccurate analysis results. One of the tests carried out in the clinical laboratory is total protein. Handling of lipemic serum can be done by several methods including centrifugation and precipitation. Precipitation is carried out with cyclodextrins which are water soluble and can form complexes with water molecules, and are effective in overcoming disturbances of lipid particles in lipemic serum. This study aimed to compare and analyze total protein levels in lipemic serum treated with alpha cyclodextrin, gamma cyclodextrin, and high-speed centrifugation. This study was conducted at Surabaya Jemursari Islamic Hospital Laboratory in October 2021-May 2022 and aimed to analyze the difference in total protein levels in lipemic serum that has been treated with 20% alpha cyclodextrin, 20% gamma cyclodextrin, and with high-speed centrifugation treatment. The method used in this study was a pretest-posttest design, involving 9 lipemic serums in total that were visually visible. Determination of this study was performed by statistical analysis using the SPSS application. The asymptotic value obtained was 0.248 for alpha cyclodextrin treatment; 0,125 for gamma cyclodextrin treatment; and 0,229 for high-speed centrifuge treatment. Since the obtained asymptotic value was >0.05. It can be concluded that there was no significant differences in the treatment of lipemic serum with the treatment of alpha cyclodextrin, gamma cyclodextrin, and high-speed centrifugation.
***Keywords**: cyclodextrin; high-speed; centrifugation; lipemic serum; total protein; precipitation*

INTRODUCTION

Serum is the part of the blood obtained after the blood clotting process and is almost commonly used as an ingredient in chemical tests. Examination samples in the form of serum that meet the requirements of laboratory tests should not appear red (hemolysis), jaundice, or cloudy (lipemic).[1] Lipemic serum is serum that becomes cloudy due to increased lipoprotein levels and accumulation of lipoprotein particles. Chylomicrons, which have the largest lipoprotein particles with a size of 70-100 nm, are the main cause of serum turbidity.[2] Lipemic serum can interfere with examination parameters that require light transmission. Turbidity in lipemic serum can affect spectrophotometric absorbance at almost all wavelengths, so that it can cause inaccurate analysis result.[3] One of the examinations carried out in a clinical laboratory using a spectrophotometric device is total protein. Total protein examination was carried out using the biuret method. If the sample used is a lipemic sample, of course it will affect the results of the examination and can cause misdiagnosis.[4] The methods used to remove fat in serum include fat extraction using organic solvents, centrifugation, and precipitation.[5]

Clinical and Laboratory Standards Institute (CLSI) recommends the use of ultracentrifugation method as a standard procedure for handling lipemic serum sample.[6] However, not all laboratories are capable or equipped with ultracentrifugation equipment due to the very low amount of lipemic samples that may be received per year.[7] According to research conducted by Castro (2018), it is stated that high speed centrifugation can replace ultracentrifugation to remove lipemics in patient serum. The study results are in line with research conducted by Goce & Brock (2011), which stated that high speed centrifugation is nearly as effective as ultracentrifugation in reducing serum lipids.

In addition to centrifugation, handling of lipemic serum can also be done by precipitation methods, one of which is using cyclodextrin. Cyclodextrins are natural compounds that are harmless and effective in overcoming the disruption of lipid particles in lipemic serum. Based on its constituent glucose monomers, cyclodextrins are divided into alpha-cyclodextrin with 6 units, beta-cyclodextrin with 7 units, and gamma-cyclodextrin with 8 units.[8]

Alpha and Gamma cyclodextrins have the capability to build inclusion complexes with molecules contained in the cyclodextrin molecular cavity so that they can be used to reduce turbidity in lipemic serum.

Cyclodextrins react with serum by precipitating interfering lipoproteins. The bond between cyclodextrin and lipoprotein is easily precipitated using centrifugation, and can produce a clear supernatant.[9] Beta cyclodextrins are not used in the treatment of serum lipids because their solubility in water is not good.[10]

Based on research conducted by Sujono (2017), it was found that total protein and urea levels tended to be lower with the addition of gamma cyclodextrin, and there was a significant difference between lipemic serum given addition of gamma cyclodextrin with lipemic serum without the addition of gamma cyclodextrin. In a study conducted by Dini (2019), there was a difference in triglyceride levels in hyperlipidemic serum treated with alpha cyclodextrin and high-speed centrifugation. The mean difference in triglyceride levels in hyperlipidemic serum

treated with alpha cyclodextrin was 273 mg/dL compared to 461 mg/dL in those treated with high-speed centrifugation.

This study aimed to compare and analyze total protein levels in lipemic serum treated with alpha cyclodextrin, gamma cyclodextrin, and high-speed centrifugation.

METHOD

The study was conducted at Jemursari Islamic Hospital Laboratory Surabaya from October 2021 to May 2022. Samples were obtained from the serum of patients who had been examined in the Laboratory of Jemursari Islamic Hospital. Samples are selected based on inclusion criteria such as lipemic samples that can be seen visually, not lysed, and not icteric. Total of 9 serum met the inclusion criteria. The use of samples as research material was approved by Jemursari Islamic Hospital ethics committee, and the patient's identity was not included in the research data.

The serum was then divided into 3 treatment groups, namely the group given 20% alpha cyclodextrin and 20% gamma cyclodextrin, with the ratio of solution and sample is 2:1. For the high-speed centrifuge treatment, the serum was centrifuged with 10.000 xg for 15 minutes. Total protein examination was performed using spectrophotometer TMS 24i Premium to determine the total protein level before and after treatment.

RESULTS

Study on the comparison of total protein levels in lipemic serum with the addition of alpha-cyclodextrin, gamma-cyclodextrin, and with high-speed centrifugation treatment has been carried out at the RSI Surabaya Jemursari Laboratory. Results of the study are presented in the form of tables with explanations such as the following:

Table 1. Average total protein levels before and after treatment

	Total protein levels before treatment (g/dL)	Total protein levels after treatment (g/dL)		
		Alpha-cyclodextrin 20%	Gamma-cyclodextrin 20%	*High-speed centrifugation*
Average	7.83	7.48	7.38	7.49

Based on Table 1, the average total protein levels in serum before treatment was 7.83 g/dl, and the average total protein levels in serum after treatment with the addition of alpha, gamma-cyclodextrin, and with high-speed centrifugation treatment, respectively, were 7.48 g/dl, 7.38 g/dl, and 7.49 g/dl.

Table 2. Average decrease and percentage decrease in total protein levels

Treatment	Average decrease in total protein levels (g/dL)	Percentage decrease in total protein levels (%)
Alpha-cyclodextrin 20%	0.35	4.46
Gamma-cyclodextrin 20%	0.45	5.74
High-Speed Centrifugation	0.34	4.43

Table 2 shows an average decrease and percentage decrease in total protein levels. The average decrease in total protein levels has a range of 0.34-0.45 g/dL, while the percentage decrease in total protein levels has a range of 4.43% - 5.74%. The highest decrease occurred in the treatment group with 20% of gamma-cyclodextrin.

Table 3. Anova one way statistical test results

	p	Conclusion
Alpha-cyclodextrin_Gamma-cyclodextrin_High-Speed Centrifuge	0.921	No significant difference

Based on the test results in table 3, the p-value obtained was 0.921 so it is concluded that there is no significant difference in total protein levels in lipemic serum that has been treated with alpha cyclodextrin, gamma cyclodextrin, and high-speed centrifuges.

Table 4. Paired t-test statistical test results

Treatment	p	Conclusion
Pre-post alpha cyclodextrin 20%	0,248	There is no significant difference
Pre-post gamma cyclodextrin 20%	0,125	There is no significant difference
Pre-post high-speed centrifugation	0,229	There is no significant difference

Based on the table 4, the p-value for each treatment was >0.05 so it can be concluded that there was no difference in the total protein levels in lipemic serum before and before the treatment with the three methods given.

DISCUSSION

This study aimed to determine the differences in serum lipemic total protein levels that treated with alpha cyclodextrin, gamma cyclodextrin, and high-speed centrifugation. Measurement of lipemic serum total protein

level using TMS 24i Premium with spectrophotometric principles and biuret colorimetric method. Total protein analysis using the biuret method with the addition of Cu2+, that will be bound to peptides, thus forming an alkaline solution that can be absorbed by wavelength.[11]

The statistically processed data in the One-Way Anova test showed that there was no significant difference in the three treatment groups in serum. The factor causing the absence of lipemic serum differences could occur because the total protein had the same properties as enzymes, which could be influenced by the degree of acidity (pH).[12] The acidity condition of the cyclodextrin solution was needed to be considered because low pH or high pH could cause protein denaturation.[13] This was in line with the research of Sharma (1990) which compared the treatment of lipemic serum with the use of alpha cyclodextrin and ultracentrifugation methods. The results of Paired Sample T-Test showed that there was no significant difference between the two lipemic serum handling techniques. There was no difference because alpha-cyclodextrin removed more lipids than mild lipemic serum ultracentrifugation, but in cases of severe lipemia, both procedures removed equivalent amounts of lipids. The study results were in line with research that conducted by Dewanti Kurnia Putri (2016), the results showed an average difference of 41.46 mg/dl with a percentage of 20.95% so that it can concluded that there was no significant difference in the use of alpha cyclodextrin with gamma cyclodextrin.

The data processed by the Paired T-test showed that there was no difference in total protein levels in lipemic serum before and after treatment in lipemic serum. The factor causing the absence of differences before and after treatment can be caused by differences in the level of turbidity of lipemic serum so that the cyclodextrin concentration used at a concentration of 20% has not been known for its effectiveness whether the concentration was able to precipitate lipids in lipemic serum at mild, moderate and severe level.[14] In addition, the incubation temperature was an important thing that must be considered because the sample needed to be incubated so that the cyclodextrin could bind lipemic to the serum. Uncontrolled temperature resulted in the formation of cyclodextrin complexes with unstable lipemic serum. The increase in temperature caused the inclusion complex to be unstable, therefore the interaction between the cyclodextrin and the guest molecule was easily separated.[15]

This study results were not in line with the study that has been done by Dini (2019). The results showed that the mean difference in triglyceride levels with lipemic serum treated with Alpha Cyclodextrin and High-Speed Centrifugation was 273 mg/dL. The statistical test results showed that there was a difference in lipemic serum triglyceride levels before and after treatment with alpha cyclodextrin and high-speed centrifugation. In the study of Rosenadia (2017), stated that the average difference in calcium levels in lipemic serum with and without the addition of gamma-cyclodextrin incubation at 23 °C was 5.47 mg/dl (30%). Paired T-Test results showed that there was a difference in serum calcium levels in lipemia with and without the addition of gamma cyclodextrin when incubated at 23 °C. This was because the temperature was one of the determining factors for the success of flocculation. In this study a temperature of 23°C was used during incubation to maximize the flocculation process. Therefore, after the centrifugation process, a clearer serum will be obtained.[16]

The addition of cyclodextrin showed a lower lipemic level, due to the cyclodextrin structure which can form complexes with lipoproteins so that cyclodextrins can precipitate disturbing lipoproteins in the serum.[17] After being given the addition of cyclodextrin, the serum was centrifuged. The serum would be separated into two parts, the upper part containing a clear supernatant and the lower part containing lipoprotein deposits that have been bound with cyclodextrin flocculants. In the treatment group with high-speed centrifugation, the serum visually also looked clearer. The serum after being treated with high-speed centrifugation was also divided into two parts. The separation was between the clear serum at the bottom with the floating lipoproteins at the top. Some of the lipoprotein deposits on the walls of the serum cup. This was because the lipoprotein particles were distributed according to density after centrifugation. Chylomicrons and VLDL were on top of the serum and formed different layers because of their low density.[18]

Study Limitation

This study was not classify the level of lipemic serum turbidity and was not involve temperature control during the incubation of the cyclodextrin solution. Strongly recommend that future studies include larger samples, and may use another parameters.

CONCLUSION

Based on the objectives and results of the study analysis, it can be concluded that there were no significant differences in the treatment of lipemic serum with the addition of alpha-cyclodextrin, gamma-cyclodextrin, and with high-speed centrifugation.

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