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Overview of Triglyceride levels in Lipemic Serum using Polyethylene Glycol (PEG) 6000

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ABSTRACT

Lipemic causes interference with wavelength and light scattering caused by the presence of lipid particles. This study aims to determine the description of triglyceride levels in lipemic serum with and without the addition of polyethylene glycol (PEG) 6000. This type of research is experimental. The research conducted was descriptive research. The samples used were lipemic serum totaling 30 samples with triglyceride levels above 300 mg/dl. The results of the data analysis showed a mild lipemic level of 22 samples with an average of 379.00 mg/dl, a moderate lipemic level of 5 samples with an average of 558.40 mg/dl, and a severe lipemic serum of 3 samples with an average of 943.40 mg/dl. Based on the results of triglyceride levels without the addition of PEG 6000, the average level is 465.33 mg/dl, while the results of triglyceride levels using PEG 6000, the average level is 243.23 mg/dl. Based on the difference in triglyceride levels before and after adding PEG 6000, namely at the mild level, the average is 215.00 mg/dl (56%), at the moderate level, the average is 261.00 mg/dl (46%), and at the severe level, the average is 212.30 mg/dl (22%). It can be concluded that there are differences in triglyceride levels in lipemic serum before and after adding PEG 6000.

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1. Introduction

Clinical laboratories are health care facilities that occupy an important position in making a diagnosis (Mahon & Lehman, 2022; McPherson & Pincus, 2021; Petersen et al., 2019). To get accurate results, the laboratory must refer to good laboratory procedures (GLP) which include pre-analytical, analytical, and post-analytical stages (Arooj et al., 2022; Chougule et al., 2022; Lenicek Krleza et al., 2019). In the pre-analytical stage, errors contribute about 61% of the total laboratory errors, one of which is the preparation

of patients before sampling in the form of blood that will become serum. For clinical chemistry examination, some parameters are influenced by food, so sampling must be done after the patient has fasted for a certain period of time, which is 8-10 hours. If this condition is not met, it will cause poor sample conditions, for example, the sample becomes lipemic. The importance of laboratory examination results as support for diagnosis, determination of treatment, evaluation of treatment results and decision making for patients, it is necessary to handle lipemic serum in order to obtain accurate examination results so that patients can be treated so that patients are treated appropriately (Lysaght et al., 2019; Mimi Sugiarti, 2021).

Triglycerides are one type of fat found in the blood. Triglycerides are the result of the body's description of fat-containing foods that have been consumed and enter the body and are formed in the liver, Triglycerides will be absorbed by the intestines after undergoing hydrolysis and enter the blood plasma and then distributed to all body tissues. Triglyceride function is to provide energy to the body from consumed calories. Normal triglyceride levels with risk classification of triglyceride levels 150 - 199 mg/dl (high limit), 200 - 499 mg/dl (high), and > 500 mg/dl (very high). High triglyceride levels will affect the sample that will be used because it can trigger the occurrence of lipemic serum (Permatasari, 2015).

Hypertriglyceridemia is an increase in triglyceride levels above normal limits. One of the risk factors for heart disease is an increase in triglyceride levels in the blood. Increased triglyceride levels can cause atherosclerosis. A condition where cholesterol accumulates in the walls of arterial blood vessels is atherosclerosis. The occurrence of atherosclerosis can result in hardening of the arteries resulting in narrowing of the arteries. Narrowed arteries can inhibit blood flow to the heart which can lead to coronary heart disease (Styawan, 2021).

Several methods can be used to purify lipemic serum: centrifugation, extraction, dilution and precipitation (Li et al., 2022; Spaggiari et al., 2019). The gold standard to purify lipemic serum is centrifugation with an ultracentrifuge, but this method requires additional equipment in the form of an expensive ultra centrifuge. Another method to purify lipemic serum can be done by precipitation method using PEG 6000. Where this PEG can bind fat and then precipitate it so that the serum becomes clear. PEG 6000 is a harmless and cheap chemical compound (Mimi Sugiarti, 2021).

PEG is used to dissolve preparations that are insoluble in water (Chakravarthy et al., 2023; Yousaf et al., 2019). When PEG is attached to other polymer molecules, it can affect the chemical properties and fat molecules. PEG binds the absorption and dissolution of a fat that is difficult to dissolve in water. Precipitation to purify lipemic serum can be done by using PEG that can bind fat. After the fat is bound, centrifugation is carried out so that the fat settles and the serum becomes clear (Sari, Hardisari & dan Sujono 2017). Surfactant is a substance added to a liquid to increase solubility, uniting two different compounds. PEG is one type of surfactant, PEG has the principle of increasing solubility. The main properties of PEG are stable, soluble in water and can bridge two different phases. So handling lipemic serum with PEG can be an alternative to precipitate lipemic serum (Alshoug, 2020; Susilawati, 2023; Yang et al., 2022).

Research by Damacker in 1980 stated that PEG with a concentration of 75g/l (7.5%) is the optimal concentration that can be used in handling lipemic serum and can be aligned with the ultracentrifugation method. According to research by Mimi Sugiarti & Eka Sulistianingsih (2021) the test results of samples precipitated with PEG 6000 1: 1 can reduce the levels of glucose, SGOT and SGPT examination values. According to Wheny Mufita Sari, Ni Ratih Hardisari & Sujono (2017) lipemic serum treated with PEG 6000 8% looks clear because the lipoproteins that cause lipemia in this serum have been bound by PEG. In 2002 WHO recommended handling lipemic serum with the addition of Polyethylene Glycol (PEG) 6000 flocculant at a concentration of 8% (Ii 2016). So in this study I used PEG 6000 at a concentration of 8%.

2. Methods

This type of research is descriptive research (Lans & Van der Voordt, 2002; Siedlecki, 2020). The place of sampling and research was conducted in the laboratory of STIKes Karsa Husada Clinic Jl. Subyadinata No.7, Jayaraga, Kec. Tarogong Kidul, Garut Regency, West Java 44151. The research time began in

December 2022 - February 2023. Triglyceride levels with lipemic serum were examined using the colorimetric enzyme method (GPO-PAP), photometer device. Reference value <150 mg/dl. The study population was lipemic serum at Kimia Farma Laboratory. The sample size taken was 30n samples. The tools used were analytical balance, measuring flask, micropipette, yellow tip, blue tip, tissue, tube, centrifuge and chemical analyzer. The materials needed in this study are lipemic serum and triglyceride reagents. How to collect data by obtaining lipemic serum from the Kimia Farma Clinical Laboratory, then conducting experiments on lipemic serum with 2 treatments, namely not adding PEG 6000 and adding PEG 6000. Data analysis in this study was descriptive, describing the research variables, presented in the form of a frequency table distribution table and calculated using the formula for the average amount equal to the amount of data divided by the amount of data.

3. Result and Discussion

This study was conducted at the Clinical Chemistry Laboratory of STIKes Karsa Husada Garut. 30 serum samples were taken from Kimia Farma Clinical Laboratory, with inclusion criteria, namely lipemic serum samples that have triglyceride levels above 300 mg/dl.

Table 1.
Frequency Distribution of Number of Samples Based on Triglyceride Levels

Color and turbidity	Triglyceride levels	Average triglyceride levels (mg/dl)	Lipemic level	Quanti ty
Milky white	300-449	379,00	Mild	22
Milky white and cloudy	500-799	558,40	Medium	5
Milky white and very cloudy	800-1800	943,33	Heavy	3

Based on table 1, it can be seen that the most lipemic samples found are mild levels of milky white which have triglyceride levels of 300-449 having an average of 379 mg/dl totaling 22 samples.

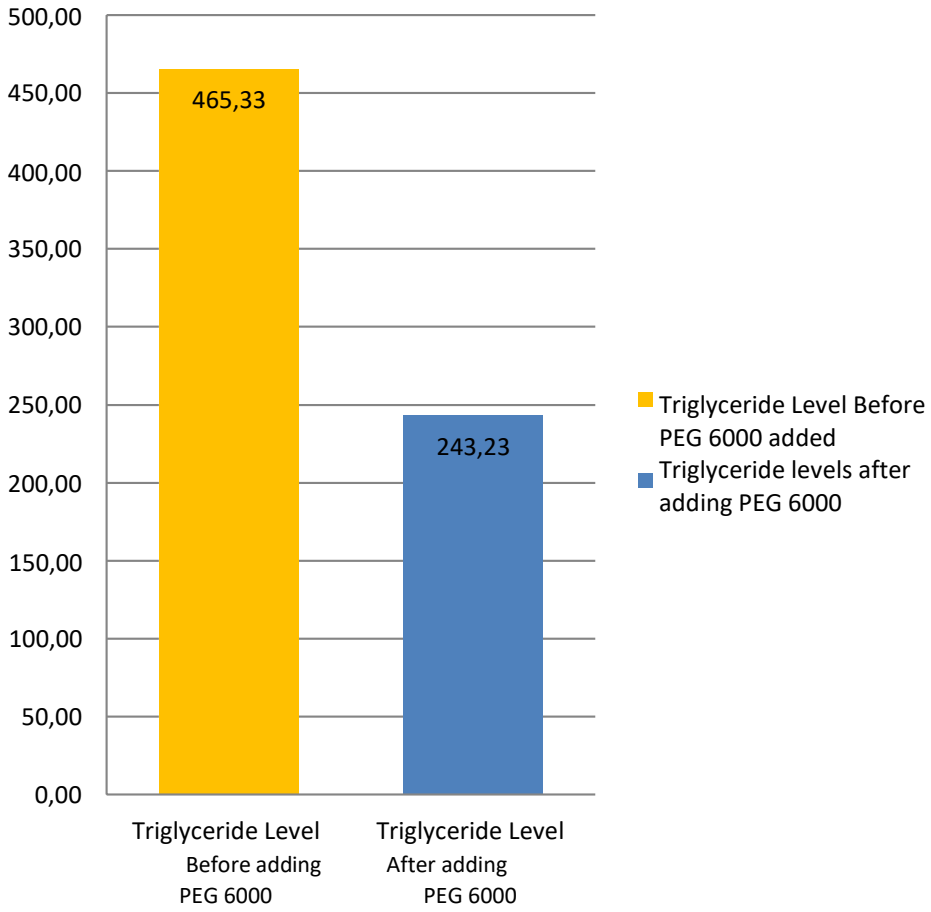

Figure 2.
Mean Triglyceride Level Examination Results With and Without PEG 6000 Addition

Examination of triglyceride levels with the addition of PEG 6000 is lower than triglyceride levels without the addition of PEG 6000 decreased by 222.10 mg/dl (52.3%), seen in Figure 4.1 bar chart of the average triglyceride levels. This is caused by PEG 6000 which can purify lipemic serum by binding protein molecules.

Table 2.
Mean Difference in Triglyceride Levels With and Without PEG 6000 Addition Based on Lipemic Levels

	Average triglyceride levels (mg/dl)		Percentage	
Lipemic level	Without PEG added 6000	With PEG added 6000	Difference in level (mg/dl)	Difference in level (%)
Mild	379,0	164,0	215,0	56
Medium	558,4	297,4	261,0	46
Severe	943,3	731,0	212,3	22

In table 2. the average difference in the results of the examination of triglyceride levels based on mild lipemic levels was 215.0 mg/dl (56%) while moderate lipemic levels were 297.4 mg/dl (46%) and severe lipemic levels were 212.3 mg/dl (22%). The results of triglyceride levels in lipemic serum with the addition of PEG 6000 8% were lower than triglyceride levels without the addition of PEG 6000 8%. The examination of triglyceride levels in this study used the GPO-PAP method. Based on table 4.1 of the 30 samples, there

were 22 mild lipemic serum samples with an average level of 379.00 mg/dl, 5 moderate lipemic serum samples with an average level of 558.40 mg/dl and 3 severe lipemic samples with an average level of 731.00 mg/dl. The cause of serum becoming lipemic is the accumulation of lipoprotein particles, especially chylomicrons and very low density lipoprotein (VLDL), so that the sample looks milky (Munawirah et al. 2019). Turbidity in lipemic serum can be seen by eye, lipemic serum can also occur due to an increase in lipoprotein concentration which causes the serum to become cloudy (Sari et al., 2017).

Precipitation to clear lipemic serum can be done by using PEG that can bind fat. After the fat is bound, it is then centrifuged so that the fat settles and the serum becomes clear (Sari, Hardisari, and Sujono 2017). PEG can reduce the surface tension of the solution, by means of polar and non-polar groups on the head and tail will bind to non-polar groups on fat, so that fat can be bound by PEG (Susilawati, 2023). Triglyceride levels above 200 mg/dl are called hypertriglyceridemia. Hypertriglyceridemia can reach 500 mg/dl, 1000 mg/dl, and sometimes even 2000 mg/dl (Sari et al., 2017). Increased blood triglycerides or hypertriglyceridemia is influenced by genes and food consumption such as carbohydrates, fats, and alcohol. LPL (Lipoprotein Lipase) enzyme activity can affect triglyceride levels, the LPL enzyme functions to hydrolyze triglycerides into fatty acids and glycerol. Low LPL activity will increase blood triglyceride levels (Schwingshackl & Hoffmann, 2013).Aktivitas fisik yang kurang dan pola makan yang salah berisiko mengalami penumpukan lemak serta trigliserida dalam tubuh. Kadar trigliserida dalam darah juga dipengaruhi oleh asupan. Asupan lemak dan karbohidrat yang berlebihan dapat meningkatkan kadar trigliserida dalam darah. Trigliserida yang tinggi dapat diatasi dengan cara mengatur asupan. Konsumsi sayur dan buah yang tinggi akanserat serta vitamin dapat menurunkan kadar trigliserida dalam darah (Watuseke et al., 2016). Pemeriksaan kadar trigliseridayang menggunakan PEG 6000 lebih rendah dari kadar trigliserida tanpa penambahan PEG 6000 yang dapat dilihat pada gambar 4.1 rerata kadar trigliserida sebelum ditambahkan PEG 6000 sebesar 465,33 mg/dl dan rerata kadar trigliserida setelah ditambahkan PEG 6000 sebesar 243,23 mg/dl. Terdapat perbedaan kadartrigliserida sebelum dan sesudah ditambahkan PEG 6000 dengan rerataselisih yang didapat sebesar 222,10 mg/dl. Hal ini karena PEG mampu mengikat absorbsi dan disolusi suatu lemak yang sukar larut dalam air serta PEG juga dapat mengikat lemak kemudianmengendapkannya sehingga serum menjadi jernih (Mimi Sugiarti, 2021).

Based on table 2. the average difference in the results of triglyceride levels at mild lipemic level was 215.00 mg/dl (56%), while at moderate lipemic level was 261.00 mg/dl (46%) and at severe lipemic level was 212.30 mg/dl (22%). In this study, triglyceride levels in lipemic serum tended to be lower after being added with PEG 6000, this is because the examination of triglyceride levels uses colorimetric principles where enzymatic changes are calculated based on color changes. Turbid lipemic serum causes the measured color intensity to be higher which makes the total protein content of lipemic serum high as well, but after adding PEG 6000 the serum becomes clearer, so triglyceride levels tend to be lower after adding PEG 6000. Based on the research conducted, the flocculation method with PEG 6000 can reduce the turbidity of lipemic serum. The higher the lipemic level in the serum, the smaller the decrease obtained because at a high lipemic level when the serum is added with PEG 6000 at a concentration of 8%, it still looks cloudy and the decrease obtained is only 22%, so a different ratio between lipemic serum and PEG 6000 is needed or a longer incubation time is needed so that PEG can bind to lipoproteins in lipemic serum. Lipemic serum that is directly examined without handling first is quite disturbing for laboratory examinations. This causes lipemic serum that is directly examined to result in false high results (Aryani, 2021). The results of the lipemic serum study using PEG 6000 look clearer than before adding PEG 6000, because the addition of PEG 6000 flocculant to the lipemic serum sample will bind to lipoprotein molecules, so that lipoproteins will precipitate after centrifugation and the serum becomes clear.

4. Conclusion

Based on the results of research that has been done with 30 lipemic serum samples, it can be concluded that: The mean examination of triglyceride levels in lipemic serum without the addition of PEG 6000 was 243.23 mg/dl, the mean triglyceride levels with the addition of PEG 6000 flocculant was 465.33 mg/d and there was a difference in triglyceride levels before and after treatment can be seen from the mean difference in triglyceride levels in lipemic serum with and without the addition of PEG 6000 was 222.30

mg/dl with a percentage difference (52.3%). For research development, it is recommended to conduct a more in-depth study involving a larger sample group and a wider variety of lipemic severity. Research can consider using different types of polyethylene glycol (PEG) with various molecular weights to better understand the role of PEG in reducing triglyceride levels. Attempt to identify the molecular mechanism behind the reduction in triglycerides following the addition of PEG 6000 in lipemic serum, which may provide additional insight into the potential clinical applications of these findings. Considering the long-term impact of PEG 6000 addition on the properties of lipemic serum and whether this reduction in triglycerides affects the risk of lipid-related diseases such as heart disease.

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