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RESEARCH

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The Effect of Triglycerides and Total Cholesterol Levels of Lipemic Plasma on The Results of HBsAg Screening Using Chemiluminescence Immunoassay

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Abstract

The results of the Immunochromatographic Method for Infectious Disease Testing (IMLTD) may vary depending on the presence of lipids in donor blood samples, potentially leading to false-negative or false-positive outcomes in hepatitis B surface antigen (HBsAg) screening. This study aimed to evaluate the effect of lipid interference on HBsAg screening using the chemiluminescent immunoassay (CLIA) method, to determine the range of triglyceride levels that may influence test results (100 mg/dL to >3000 mg/dL), and to assess practical lipid removal methods to ensure blood safety. An experimental laboratory study was conducted using two sample groups. The first group consisted of six bags of non-lipemic, HBsAg-reactive plasma that were rendered lipemic by the addition of Lipofundin. The second group included 25 bags of lipemic donor plasma that were non-reactive in the initial IMLTD screening and subsequently subjected to lipid removal treatments. Lipid reduction was performed using centrifugation, polyethylene glycol (PEG), and diethyl ether. The results showed no significant differences in HBsAg values before and after Lipofundin addition, indicating that lipemia did not affect HBsAg detection by the CLIA method. However, lipid removal using centrifugation, PEG, and diethyl ether significantly reduced triglyceride and total cholesterol levels ($p < 0.05$) in lipemic samples that interfered with CLIA analysis. In conclusion, lipemia does not directly affect HBsAg screening results using the CLIA method; however, lipid removal procedures are effective and necessary for managing highly lipemic samples to maintain the accuracy and reliability of blood screening tests.

Keywords: Chemiluminescence Immuno Assay, HBsAg Screening Test, Triglyceride, Total Cholesterol.

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1. INTRODUCTION

Blood transfusion services, as one of the health services, should provide sufficient, safe, easily accessible, and affordable blood for the community to save lives. One of its tasks is to ensure that the safe blood is screened for the Transfusion-Transmitted Infection (TTI) of the blood donor's sample (Negash et al., 2019). In Indonesia, Blood banks conduct TTI screening tests for HIV (Anti-HIV 1 and 2), Hepatitis B (HBsAg), Hepatitis C (anti-HCV), and Syphilis (Anti-TP) using assays validated for high sensitivity and specificity. Various substances in the sample can interfere with the immunoassay, so the sample must meet several essential requirements. Experts define interferences as the effects of substances that alter assay results, usually based on their concentration. Some types of interferences that can change the results, such as hemolysis, can increase or decrease the level of analytes, affecting tests for folate, vitamin D, and thyroid antibodies (Martin Calderon, 2023). Jaundice, high bilirubin levels, can interfere with hormone levels. And protein testing, leading to incorrect results in creatinine and total protein tests (Maksymyuk et al., 2023), and Lipemia, elevated triglyceride concentrations, can distort measurements for various analytes, including BNP and homocysteine (Karin et al., 2023).

Laboratories categorize in-sample interferences as endogenous or exogenous. The endogenous interferences arise from interactions within the sample, affecting analyte concentration or antigen-antibody binding, generally caused by hemolysis, lipemia, jaundice, or additives in the blood sample. Exogenous interferences occur due to external substances introduced in vivo or in vitro, such as radioactive or fluorescent compounds, drugs, herbal supplements, or problems during sample transportation and storage (Ghazal et al., 2022).

Lipemia is one of the most common disorders of the sample state, a condition in which the amount of lipids increases in the blood and usually occurs after eating. Hence, the blood sample appears like "milk". Lipemic serum or triglyceride levels and/or high cholesterol can provide incorrect results in some immunological examination tests because of antigen binding disorders, even though antibodies have been attached to the tube surface (Prendes et al., 2023). Lipemic conditions do not cause transfusion reactions. However, excessive lipemia can interfere with the results of the TTI screening test (Gupta et al., 2022).

Lipids are all compounds that are not soluble in water but are soluble in organic solvents. The main lipids in the blood are triglycerides, cholesterol esters, phospholipids, and small amounts of free fatty acids. Lipids do not dissolve in plasma, so lipoproteins must bind to them to enable their dissolution." Lipemia is a sample turbidity caused by the accumulation of lipoprotein particles, with chylomicrons being the largest particles that contribute the most to turbidity. Not all classes of lipoproteins contribute equally to turbidity, as HDL, LDL, and small VLDL do not result in lipemic samples" (Prendes et al., 2023). The largest particle, a kilomicron (90% triglycerides and 5% cholesterol), with a sample size of 70-1000 nm, has the most potential for causing sample turbidity.

The mechanism of interference of lipids in plasma can be physical or chemical. Physical mechanisms interfere with the photometric reading because lipoproteins in the sample will absorb light. Also, at the pipetting step, chylomicron and VLDL (Very Low Density Lipoprotein) particles that have a lower density will be at the top of the tube to form a layer that will be taken as a sample, so that it can give invalid inspection results. Mechanism chemistry can disrupt antigen-antibody reactions because lipoproteins block the antigen-antibody binding area. The mechanism of physical and chemical interference can be eliminated by centrifugation during sample preparation (Soh et al., 2022b). The recommended procedure for removing lipids is ultracentrifugation at 100,000 - 2,000,000 x g speeds. However, this centrifuge is not available in a large number of laboratories. Studies have tested agents such as LipoClear and 1,1,2-trichlorotrifluoroethane, but results indicate that these agents can interfere with specific analytes, limiting their suitability for routine use (Castro-Castro et al., 2018). The studies have shown that high-speed centrifugation consistently reduces lipemia for various

analytes, including albumin and creatinine, while lipid clearing agents show mixed results (Gardiner et al., 2020).

In Indonesia, according to Blood Service Standards in the Minister of Health Regulation No. 91 of 2015, the standard for TTI screening test in a blood center is the Enzyme Immuno Assay (EIA) method. EIA methods, including ELISA, effectively identify TTIs, with studies reporting prevalence rates of 3.3% for various infections among blood donors (Jacob et al., 2023). Currently, The Blood Center (UTD) uses several brands of EIA and Chemiluminescence (CLIA) assays. Still, only a few manufacturers claim their tests can detect TTI in samples with triglyceride concentrations ranging from below 1,000 mg/dL to less than 3,000 mg/dL. Currently, most UTDs do not observe the types and levels of lipids contained in lipemic samples, and they directly reject these samples without doing the TTI screening test, which will impact the availability of blood stock.

Analysis results, leading to misdiagnosis and unnecessary treatment (Berman & Hayes, 2024). Lipemic samples may cause increased fragility of red blood cells, exacerbating haemolysis during collection and storage (Winter et al., 2022). In other UTDs, they do the TTI screening test using these lipemic samples without any specific treatment, so we are not sure whether these results are false negatives or false positives because of the interference of lipids in the plasma with the assay. From observations in some UTD, blood donors often present with lipemic plasma. In UTD PMI DKI Jakarta, 2.415 donor blood bags were lipemic from 234.539 donor bags collected (1%) in 2017.

The prevalence of lipemic samples in Indonesia significantly impacts Immunoglobulin Titer Test results, particularly in HBsAg screening tests. Lipemia can interfere with the accuracy of serological tests, leading to false-negative or false-positive results. Lipemic samples can cause turbidity, affecting optical density readings in the assay, leading to misinterpretation of HBsAg results. Lipids can interfere with antigen-antibody interactions crucial for accurate TTI results, often requiring additional testing or sample processing. This issue becomes more challenging due to the high prevalence of Hepatitis B in Indonesia, which varies across regions and demographic groups. So, in this study, we want to know the impact of lipemic samples on the TTI results, especially the HbsAg Screening test, because specific studies reported HBsAg favorable rates of 0.82% to 3.61% among blood donors, with higher rates in men and younger age groups (Hippy & Ukma, 2022). Researchers determined the prevalence of reactive and non-reactive hepatitis B virus infection in the West Lombok Indonesian Red Cross unit. The results showed that most donors were men, with the highest number found in the 25–44-year age group (4,342 individuals) (Kisniarti et al., 2023). Transfusion-transmitted infection (TTI) screening test in blood centers is the enzyme-linked immunosorbent assay (EIA) for anti-Treponema Pallidum (TP), as demonstrated by its wide use in routine blood donor screening in China (Fu et al., 2020). This study aimed to evaluate the effect of lipid interference on HBsAg screening using the chemiluminescent immunoassay (CLIA) method, to determine the range of triglyceride levels that may influence test results (100 mg/dL to >3000 mg/dL), and to assess practical lipid removal methods to ensure blood safety.

2. RESEARCH METHOD

The researchers conducted the study from July to December 2018 at UTD PMI DKI Jakarta and UTD PMI Bekasi. The researchers conducted this experimental study using six non-lipemic reactive HBsAg plasma bags. They modified them into lipemic samples using Lipofundin, and the second sample group was 25 lipemic non-reactive for all parameters in the TTI Screening test (HbsAg, anti-HCV, anti-HIV, anti-TP). The researchers treated the samples to remove the lipids in the plasma. They measured triglyceride, total cholesterol, and HBsAg levels in each sample before and after the treatment. The first group is six non-lipemic plasma

bags with HBsAg reactive and anti-HIV, anti-HCV, and syphilis are non-reactive. The reactive plasma HBsAg non-lipemic, measured levels of triglycerides and total cholesterol by the photometric method, and quantitative HBsAg testing by the CLIA method. The researchers conducted an experimental test by adding a certain amount of Lipofundin to the plasma to achieve triglyceride levels according to the categories. The researchers said Lipofundin to achieve triglyceride levels that met the criteria set for the study.

The second group that is 25 bags of lipemic plasma with HBsAg, anti-HIV, also anti-HCV Syphilis is non-reactive, the lipemic plasma, measured levels of triglycerides and total cholesterol by the photometric method and quantitative HBsAg testing the CLIA method too and In the final treatment, the researchers performed high-speed centrifugation at $21,855 \times g$ for 15 minutes. Referring to the study by Dimeski et al., they treated 25 non-reactive lipemic HBsAg samples by adding 8% PEG 6000, mixing them in a 1:1 (v/v) ratio, and incubating the mixture at 4°C for 30 minutes in a refrigerator. After incubation, they centrifuged the samples at $1,000 \times g$ for 10 minutes at 4°C . Studies show that incorporating PEG can lead to better recovery rates of viruses such as HIV, HBV, and HCV, which is crucial in transfusion safety (Dean et al., 2018). PEG 6000 functions as a flocculant by promoting lipoprotein aggregation, allowing the aggregated lipoproteins to be separated from the serum. Research shows that 8% PEG is effective for serum with triglyceride levels above 400 mg/dL, enhancing Ranto et al. (2024). The results examined were supernatants multiplied by a 2x dilution factor and extracted with diethyl ether 3 mL into 1 mL of lipemic plasma samples, shaken 40 times for 20 seconds, then centrifuged at 2000 rpm for 5 minutes. The results examined were supernatants.

The Quantitative HBsAg screening test uses the chemiluminescence immunoassay (CLIA) method to quantitatively determine Hepatitis B surface antigen (HBsAg) in human serum or plasma samples using the M5050 Liason® XL immunoassay tool. The photomultiplier device will measure illumination as a Relative Light Unit (RLU). If the concentration of the specimen is between the values of 0.050-150 IU/mL or greater than or equal to 0.05 IU/mL, the research material is considered reactive.

The researchers analyzed the data in this study using SPSS version 23.0 with the Kruskal-Wallis test because this test was a non-parametric test aimed to determine whether there were differences in the effect of adding lipofundin into the HBsAg value, where the independent variables were more than two categories and with the ANOVA test to see the average difference in each lipid removal treatment.

3. RESULTS AND DISCUSSION

The first group, non-lipemic plasma that was reactive to HBsAg, was treated with the addition of lipofundin to make the sample appear lipemic and made into seven criteria for triglyceride levels, namely, with triglyceride levels of 100 - 499 mg/dL, 500 - 999 mg/dL, 1000 - 1499 mg/dL, 1500 – 1999 mg/dL, 2000 - 2499 mg/dL, 2500 - 2999 mg/dL, and > 3000 mg/dL. Blood centers (UTDs) in Indonesia developed these seven criteria based on the detection capability of the HBsAg screening test reagent that uses the CLIA method., which states that it has a limit of detection of HBsAg in samples with triglyceride levels < 1,000 mg/dL, < 2,000 mg/dL and < 3,000 mg/dL and also taken from the results of the lipemic donor blood examination report in 2017 at PMI DKI Jakarta. Table 1 presents the measurement results before and after the addition of Lipofundin.

Table 1. HBsAg Measurement Before and After Lipofundin Addition.

Criteria for triglycerides	HBsAg criteria	Average HBsAg Value (IU/mL)		Kruskal-Wallis Test
		Before the Addition of Lipofundin	After the Addition of Lipofundin	
100 - 499	Low	2.10 ± 2.24	2.12 ± 2.23	p= 0.970
500 - 999			2.18 ± 2.33	

1000 - 1499			2.15 ± 2.30	
1500 - 1999			2.11 ± 2.25	
2000 - 2499			2.08 ± 2.22	
2500 - 2999			2.08 ± 2.22	
> 3000			1.83 ± 1.93	
100 - 499			84.25 ± 75.92	
500 - 999			84.00 ± 76.21	
1000 - 1499			84.00 ± 76.21	
1500 - 1999	Moderate	84.00 ± 76.21	83.50 ± 76.79	p= 0.982
2000 - 2499			84.00 ± 76.21	
2500 - 2999			83.50 ± 76.79	
> 3000			83.88 ± 76.35	
100 - 499			150	
500 - 999			150	
1000 - 1499			150	
1500 - 1999	High	150	150	p= 1.000
2000 - 2499			150	
2500 - 2999			150	
> 3000			150	

Note: The researchers present the data as mean ± standard deviation (SD).

Table 1 showed no significant difference in HBsAg levels between before and after the addition of lipofundin, based on the Kruskal-Wallis statistical test, with a P-value > 0.05 in all HBsAg criteria. Researchers have not identified any simple chemicals that can mimic lipemic samples' physical or chemical properties of lipemic samples. Manufacturers of intralipid reagents or the equivalent, as the researchers used in this study, were using Lipofundin to simulate lipemia/turbidity. Researchers use Lipofundin, a synthetic, sterile, non-estrogenic fat emulsion for intravenous administration, to simulate lipemic samples by adding it to serum or plasma. (1) The particles in lipofundin are equivalent in size, 200 to 600 nm (mean ≈ 345 nm), losing the range of values for large VLDL (60-200 nm) and the lower and upper ranges for kilomicros (70-1000 nm). Samples with lipofundin addition do not mimic the lipemic sample perfectly. However, the Clinical and Laboratory Standards Institute (CLSI) uses 20% Intralipid (or equivalent, Lipofundin 20%) in conducting experiments.

This research is possible because the tools and test material for screening HBsAg have ingredients that can dissolve lipids. In Quantitative Liaison® Murex HBsAg reagent, some detergents are likely to dissolve lipids at the time of examination, so that the results of HBsAg levels do not change, which means the specificity and sensitivity of the device are reasonable, with 100% sensitivity and 99.5% specificity.

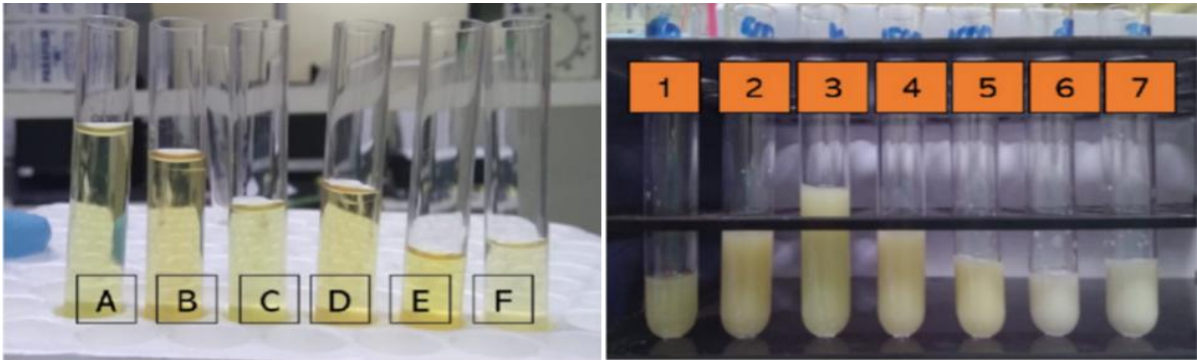


Figure 1. (Left) Experiment before the addition of lipofundin (A and B) light HBsAg reactive non-lipemic samples, (C and D) medium HBsAg reactive non-lipemic samples, (E and F) high

HBsAg reactive non-lipemic samples. (Right) After lipofundin addition (1), triglyceride levels (100 - 499) mg/dL, (2) triglyceride levels (500 - 999), (3) triglyceride levels (1000-1499) mg/dL, (4) triglyceride levels (1500-1999) mg/dL, (5) triglyceride levels (2000-2499) mg/dL, (6) triglyceride levels (2500 - 2999) mg/dL, (7) triglyceride levels >3000 mg/dL.

Figure 1. In this experimental study, the researchers treated the sample by adding Lipofundin after confirming that each sample met the seven criteria. The researchers examined HBsAg levels before and after adding Lipofundin. In the Liaison® Murexreagent HbsAg Quantitative, there is a detergent that is likely to dissolve lipids at the time of examination, so the results of HbsAg level do not change, which means the specificity and sensitivity of the tool are reasonable, with sensitivity 100% and 99.5% specificity (Burdino et al., 2014). Experts believe that lipids in donated blood cause various analytical problems, including impaired immunological measurements. The results of this study were not significantly different from the HbsAg filter test results between before and after the addition

Of Lipofundin. It is better to perform the IMLTD filter test on the sample to reduce lipemia. Researchers use mechanical or chemical methods to minimize errors in the IMLTD filter test results.

Interference is a common problem in in vitro diagnostic testing. Experts believe the presence of lipids in blood intended for donation produces various analytical problems. One of which is immunological measurement disorders, the results of which, in this study, showed no significant differences in screening test HBsAg results before and after the addition of lipofundin. The detection limit also applies to triglyceride levels from the test reagents stated in the instructions for using reagents, which is <3000 mg / dL according to the provisions set by CLSI, USA, with document number EP17-A, so that the blood component of the test results filter TTI can be declared safe according to the results of the examination. Recent progress in biosensor technology has demonstrated the ability to detect triglyceride levels effectively within this range. For example, a study by Pliego-Sandoval highlights the development of a low-cost enzymatic biosensor that can detect triglyceride levels without requiring sample dilution, achieving a detection range from 7.67 mg/dL to 58.83 mg/dL, demonstrating the potential for accurate measurement at lower concentrations (Pliego-Sandoval et al., 2023). This interpretation is critical as it shows that although the CLSI detection limit is <3000 mg/dL, new technologies can provide reliable readings at much lower levels, improving diagnostic capabilities.

However, Researchers should perform the TTI filter test on lipemic samples for better evaluation. Reduction mechanically or chemically to minimize errors in the TTI filter test results (Soh et al., 2022). The lipemic reduction recommended by the CLSI is ultracentrifugation at 40,000 xg for 30 minutes or high-speed centrifugation of 21,855 xg for 15 minutes. These methods effectively reduce lipid levels. In addition, researchers can extract lipids using organic solvents. The Clinical and Laboratory Standards Institute (CLSI) recommends using mechanical or electromechanical methods for all samples containing substances that interfere with light transmission, thus including lipids. Implications of the research in the world of health (specifically blood banks): 1) Certainty of the Validity of HBsAg Screening Test in Lipemic Samples. This study shows that lipemia (with triglyceride levels up to >3000 mg/dL) does not affect the results of the HBsAg screening test using the CLIA method. This interpretation assures the validity of HBsAg screening results in blood donors with lipemic samples, so there is no need to disqualify donors immediately because of lipemic plasma. Avoiding the wastage of blood bags due to the false assumption that lipemia causes invalid results. 2) Improving operational efficiency at the blood bank is possible because data show that lipemia does not interfere with HBsAg results. As a result, staff can reduce the time and cost associated with retesting or discarding samples. Laboratory personnel should redirect procedures for handling lipemic samples only if other tests (e.g., liver enzymes, lipid panel) are

compromised, not for HBsAg testing. 3) Utilisation of Sample Clarification Techniques. Studies have also shown that treatment with Centrifugation, PEG (Polyethylene Glycol), and Diethyl Ether.

Mechanical and Chemical Lipid Removal of Triglyceride and Total Cholesterol Levels

Second group, namely twenty-five samples of non-reactive lipid plasma HBsAg, was given three different treatments, namely high-speed centrifugation, the addition of 8% PEG 6000 and the addition of diethylether to see the percentage of decrease and the total triglyceride and cholesterol levels were analyzed with the Anova statistical test to see the difference in each treatment group. The measurement results of High Speed Centrifugation, Table 2, show the effect of 8% PEG 6000 and diethyl ether addition on triglyceride levels.

Table 2. Effect of High Speed Centrifugation, Addition of PEG 6000 8%, and Addition of Diethyl Ether on Triglyceride Levels.

	Average Triglyceride Level (mg/dL)			Anova Test
	Before Treatment	After Treatment	% difference decreased	
High Speed Centrifugation	1321	755	43%	p = 0.001
PEG 6000 8%	1321	871	34%	p = 0.006
Dietyl ether	1321	335	75%	p = 0.000

From this study, Table 2 illustrates the significant percentage reduction in triglyceride levels in each treatment, with the most influential treatment being the addition of diethyl ether (75%), high-speed centrifugation (43%), and the addition of PEG 6000 8% (34%). Table 2 also shows that these three treatments provide significant differences with a p-value of <0.05. The procedure utilized in this study, using a high-speed micro-centrifuge, effectively reduces lipid levels and offers a suitable alternative for ultracentrifuged samples to provide accurate results (Dimeski & Jones, 2011). The study investigates the impact of lipemia on routine biochemical tests, revealing that lipemic samples can lead to significant laboratory errors and emphasizing the need for standardized protocols to manage such samples. It highlights the varying effects of mild, moderate, and severe lipemia on biochemical parameters, indicating that laboratories should verify manufacturers' claims regarding lipemic interference and establish decision limits for sample rejection (Arulvijayavani et al., 2023).

Table 3. Effect of High Speed Centrifugation, Addition of PEG 6000 8%, and Addition of Diethyl Ether on Total Cholesterol Levels

	Average Triglyceride Level (mg/dL)			Anova Test
	Before Treatment	After Treatment	% difference decreased	
High Speed Centrifugation	370	224	39%	p = 0.002
PEG 6000 8%	370	310	16%	p = 0.038
Dietyl ether	370	47	87%	p = 0.000

Table 3 illustrates the percentage reduction in total cholesterol levels in each treatment, where the most influential treatment is the addition of diethyl ether (87%), high-speed centrifugation (39%), and the addition of PEG 6000 8% (18%). Table 3 also shows that these three treatments provide significant differences with a P value of <0.05. The results conclude that diethyl ether removes a larger amount of lipids compared to high-speed centrifugation and the addition of 8% PEG 6000. By its nature, lipids are soluble in organic solvents.

In the United States and other European countries, Fat particles suspended in turbid plasma can interfere with analytical methods in the IMLTD screening test, thereby supporting the exclusion of such plasma. In contrast, in Canada, lipemic blood is considered safe and is

suitable for producing blood components. The lipemic reduction recommended by CLSI is 40,000 xg ultracentrifugation for 30 minutes, or centrifugation of 21,885 xg for 15 minutes, effective for reducing lipid levels. CLSI currently supports using mechanical or electromechanical methods in all samples containing substances that interfere with light transmission, including lipids. The second group of twenty-five non-reactive lipemic plasma samples was HbsAg given three different treatments, namely, high-speed centrifugation, The researchers added an 8% PEG 6000 solution and diethyl ether to the samples as part of the treatment process. To see the percentage decrease, the triglyceride and total cholesterol levels were analyzed with the ANOVA statistical test to determine the difference among the groups.

Lipids are relatively non-polar molecules, so lipids can be withdrawn from the sample using relatively non-polar solvents; only the non-polar molecules in the sample dissolve while the polar ones do not.^{3,4} Some studies use diethyl ether as a solvent because of its low boiling point, relatively non-toxic, and non-polar nature. Lipids in human samples have polar and non-polar regions, such as triglycerides, so when added to diethyl ether, there are two separate layers. Adding energy from outside is done by shaking to dissolve it. Hence, the two solutions can mix. Centrifugation separates the diethyl ether from the sample once the centrifugal force diminishes. Diethylether carries triglycerides and soluble cholesterol in the sample, so that the levels of triglycerides and total cholesterol in the sample fall.

This study showed samples after high-speed centrifugation containing two layers; the lipids were at the top because of their specific lower gravity, and then the supernatant was taken to examine triglyceride and total cholesterol levels. Polyethylene glycol binds the lipids in lipemic plasma, enabling their precipitation. Centrifugation then removes the lipid complexes, resulting in clear plasma. Effect of High Speed Centrifugation, 8% PEG 6000 Addition, and Addition of Diethyl Ether to HBsAg Levels

Effects of High Speed Centrifugation, Addition of PEG 6000 8% and Addition of Diethylether to HBsAg Levels, we can see in Table 4 shows that the results of HBsAg examination of Quantitative Methods on samples before treatment and after three treatments mechanically and chemically, namely high speed centrifugation, addition of 8% PEG 6000 and addition of diethylether, there is no change in the results of the examination, which still shows Non-Reactive results that is <0.03 IU/mL. This treatment shows that HBsAg filter test instruments and reagents currently used in UTD are good, with no false negative results found. The limitation of this study is that there were no lipemic samples whose HBsAg filter results were reactive, so they could not determine whether HBsAg levels in the lipemic samples changed after three treatments to remove lipids, whether HBsAg levels did not change, or whether HBsAg levels changed to be higher than before.

Table 4. Results of the HBsAg Quantitative Method.

Criteria for triglycerides	HBsAg level (IU / mL)					Information
	Total	Early	High Speed Centrifugation	PEG 6000 8%	Diethylether	
100 - 499	5	<0.03	<0.03	<0.03	<0.03	Non Reactive
999 - 1499	5	<0.03	<0.03	<0.03	<0.03	Non Reactive
1500 - 1999	5	<0.03	<0.03	<0.03	<0.03	Non Reactive
2000 - 2499	5	<0.03	<0.03	<0.03	<0.03	Non Reactive
2500 - 2999	5	<0.03	<0.03	<0.03	<0.03	Non Reactive
> 3000	5	<0.03	<0.03	<0.03	<0.03	Non Reactive

Table 4 shows that the results of the HBsAg Quantitative Method in the sample before treatment and after three treatments were mechanically and chemically, namely high speed centrifugation, addition of PEG 6000 8%, and addition of diethyl ether, no change in

examination results, ie, non-reactive results, ie, < 0.03 IU/mL. These results mean that the HBsAg filter test equipment and reagents currently used in UTD are good, with no false

Negative results found. This study did not identify any lipemic samples with reactive HBsAg screening test results, which limits its findings. Researchers can further develop future studies, including: 1) Effect of Lipemia on Other Infection Screening Tests. For example: Anti-HCV; Anti-HIV; Syphilis (TPHA/CMIA) to assess whether lipemia also affects the results of other transfusion-transmitted infection tests using CLIA/ECLIA/CMIA. 2) Validation of Clarification Techniques for Other Blood Components. Further studies could examine the effects of PEG, centrifugation, or diethyl ether on the stability of haemoglobin, red blood cells (RBCs), and platelets. This research aims to determine whether this procedure is safe to apply to blood units that will be used for transfusion, not just to test samples.

4. CONCLUSION

This study reveals a significant association between prediabetes and prehypertension with reduced vascular elasticity. Individuals with prediabetes had a 1.6-fold greater risk of having suboptimal vascular elasticity than those with normal fasting blood glucose levels. Similarly, individuals with prehypertension had a 1.5-fold greater risk than those with normal blood pressure. Notably, individuals with both prediabetes and prehypertension faced a 3.5-fold greater risk of suboptimal vascular elasticity compared to those with normal fasting glucose and blood pressure.

These findings underscore the importance of early identification and intervention in individuals with preclinical metabolic alterations. To maintain vascular elasticity and reduce long-term cardiovascular risk, individuals with prediabetes, prehypertension, or both are strongly encouraged to adopt a healthy lifestyle. This includes engaging in regular physical activity and limiting the consumption of foods high in sugar and salt.

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