COMPARISON OF TOTAL BILIRUBIN LEVELS IN SERUM WITHOUT LIGHT EXPOSURE DELAYED FOR 1 HOUR AND 3 HOURS

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ABSTRACT

The total bilirubin test is one of the laboratory tests to determine the function of the liver and bile ducts. Impaired liver function can be shown by hemolytic anemia, liver cirrhosis, hepatitis, and hepatitis carcinoma. This study aims to determine the comparison of total bilirubin levels in serum without light exposure that is delayed for 1 hour and 3 hours. The method in this study uses a quantitative approach with a type of experimental research of *Cross sectional* design. The types of data used are primary data and secondary data. The sampling technique uses *the purposive sampling* technique, with a sample number of 10 samples. The results showed that the average value of total bilirubin examination in serum (immediately checked) with an average result value of 0.514 mg/dL, while in serum it was delayed for 1 hour with an average result of 0.513 mg/dL and serum was delayed for 3 hours with an average value of 0.511 mg/dL and the result of the value (significance) > from *alpha* (0.05) or (sig > 0.05) then Ho was accepted and Ha was rejected which means There was no difference in the results of total bilirubin levels in serum without light exposure that was delayed by 1 hour and 3 hours.

Keywords: Total Bilirubin, No Light Exposure, Delayed

INTRODUCTION

Clinical Laboratory Services are the filtering part of health services which is one of the supporting factors for diagnosing enforcement. The Clinical Laboratory needs to be one of the parts in the hospital or health service that needs attention. Based on the Indonesian Minister of Health Regulation number 43 of 2013. The clinical laboratory serves various types of examinations, including blood tests, clinical chemistry, clinical microbiology, clinical parasitology, clinical immunology, clinical pathology, anatomical pathology and several other examinations that are still related to humans.

One of the examinations in the clinical laboratory is a total bilirubin examination, This examination aims to determine the condition of liver function and bile ducts.

The bilirubin examination process is carried out through three stages, namely pre-analytical, analytical, and post-analytical [25]

A factor that affects the total bilirubin examination is the delay in examination. Based on the experience that has been carried out during the clinical learning practice in one of the clinical laboratories in Gorontalo, when going to conduct a clinical chemical examination, there is a delay because the samples to be examined are not enough or not too many. The delay of the serum is carried out for no more than 30 minutes. In addition, based on a survey or interview from one of the laboratory officers at Toto Kabila Hospital, he also said that there was a delay in the examination serum if the sample to be examined was not much, but in the quotation mark the sample had

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been centrifuged and had been placed in a cup.

In this study, blood samples were used on students because, after a survey was conducted on one of the laboratory officers, adult patients who will undergo a total bilirubin examination are very rare. Based on a survey from the laboratory, a total bilirubin examination is carried out if the values of SGOT and SGPT are high or there are indications that the body is yellow.

Some circumstances require the bilirubin test to be postponed. One of the main causes of sample handling problems is the inability of the analyst to handle the sample for bilirubin examination.

Human error (because the sample was not examined immediately) or unstable total bilirubin results due to light exposure are additional causes of this. As a result, lighting has an impact on the stability of total bilirubin levels in the serum, so the examination should consider lighting [1]

However, checking total bilirubin levels can be done without losing information in some circumstances. Because it is very important to ensure that the number of samples to be examined remains low. In certain situations, high-intensity light can reduce bilirubin levels [1]

Light can damage unstable bilirubin serum. Blue light can bind to free bilirubin and lower its levels. Total serum bilirubin levels are affected by exposure to lamp light in the laboratory. exposure decreased by 25% in one hour and by 25% in three hours [1] To ensure that the serum remains stable, the bottle or tube should be wrapped in black paper or foil. After that, store it in the refrigerator or at a low temperature. People who are exposed to light have lower levels of bilirubin than people who are not exposed to light [11]

Showed that the results of serum delay research can reduce bilirubin levels, but the correct handling of samples during the sample delay process can minimize the occurrence of a decrease in bilirubin [13]

Based on previous research, serum exposed to light has a significant effect on reducing bilirubin levels compared to serum that is not exposed to light. Therefore, postponing the examination without light exposure is important to be researched because it can be used as a recommendation or reference for laboratory personnel if they want to postpone a total bilirubin examination.

The liver receives blood flow from the port system, and the hepatic arteries carry blood flow to the liver from the systemic circulation. The liver is made up of many lobules that are visible only with a microscope and have a similar shape. Kupfer cells, sinusoid channels that include the vascular endothelium, and hepatocytes are structural components [9]. The liver is made up of many lobules that are similar in shape and can only be seen with a microscope. Its structural components include hepatocytes, kupfer cells, and sinusoid ducts that include the vascular endothelium [9]

Some of the important substances that help the liver function are the synthesis of glucose and glycerol. In addition, this organ has the ability to help metabolize different types of fats and proteins. The liver not only controls the metabolism of carbohydrates and fats, but also helps cleanse the body. According to [3]

The liver is located in the abdominal cavity, between the pelvis and the chest. The organ has three surfaces: posterior, lower, and upper. The liver is a large gland that is agfragmated by twisted ligaments, the left triangle is several ligaments that connect the liver to the abdominal wall and agglomerate it. The liver also consists of portal blood vessels, bile ducts, and liver arteries. There is a small pear-shaped cage under the heart. The pacifier cage is responsible for storing the liver pacifier fluid [3]

Yellow skin color is often associated with the appearance of pale and/or dull skin, which is considered a barometer of poor health. Incidentally, the appearance of dull skin can be exacerbated by conditions that generally lead to increased inflammation and oxidative

stress, such as sleep deprivation, acute and/or chronic mental or physical stress, and poor diet. However, it is not fully understood what factors cause the appearance of yellow skin [2]

Bilirubin is a metabolite of dark red blood cells that is mostly produced in the spleen, bone marrow, and/or liver. Bilirubin is transported in the bloodstream, mostly in a tightly bound form to albumin. Free or unbound bilirubin is reported to have the ability to diffuse out of blood vessels and into tissues, where it can act favorably as an antioxidant. However, bilirubin can also be cytotoxic to cells [2]

After 120 days, the reticulondothele system breaks down erythrocytes into heme and globin. Then heme is oxidized by releasing iron and carbon monoxide into biliverdin, and globin is reduced to unconjugated bilirubin or indirec bilirubin [9]

Unconjugated bilirubin is combined with liver glucuric acid during the enterohepatic cycle to produce conjugated bilirubin, a gut gamma-glucuronidase bacterium hydrolyzes conjugated bilirubin [9]

High serum bilirubin levels can cause ecteritis. One of the signs of liver dysfunction is an increase in serum total bilirubin levels. If your liver health is good, your total bilirubin levels may be normal. Hepatobilier and erythropoietic are activated by bilirubin. This is done to identify jaundice appeals and track the progression of the disease. The total amount of bilirubin in the blood is determined through a total bilirubin test. A person may experience liver problems if they have high levels of total bilirubin [8]

This is done to assess the liver's bilirubin excretion ability; Failure of bilirubin excretion causes total serum bilirubin levels to increase. Jaundice by place can be prehepatic, hepatic, and posthepatic. The increase in liver enzymes is caused by the disease, which usually causes rapid jaundice. Because liver cells cannot excrete conjugated bilirubin into the bile ducts, bilirubin increases during hepatic. This can occur due to damage to liver cells or due to a barrier that exists inside or outside the liver bile ducts [9]

The principle of the VOX method is through the action of ionic vanadic acid at pH 3.0 bilirubin is oxidized to dehydrobilirubin and the absorption decreases at 450 nm barcompared straight to the total bilirubin concentration (Mindray Total Bilirubin Kit SOP)

Mismatch between laboratory test requests (pre-analytical), analysis, and reporting and interpretation of results is known as laboratory error [13]

RESEARCH METHODS

This study uses a quantitative approach, which aims to determine the comparison of the results of the total bilirubin madar examination in the tanoa derum of light exposure whose examination is delayed for 1 and 3 hours.

This type of research is experimental research and uses *a Cross sectional research design*. It will be held in June-July 2024 at the Laboratory of Toto Kabila Hospital, Bone Bolango Regency.

Primary data is generated from the results of examinations conducted by researchers. Secondary data is generated from the supporting literature used. The results of the bilirubin examination used *the* BS-380 mindray tool. All students of the Bina Mandiri Gorontalo University health analyst study program class of 2021. The sampling technique uses *the purposive sampling* technique, with a sample number of 10 samples.

Serum independent variable without light exposure was delayed for 1 hour and 3 hours. Dependent Variables of total bilirubin *levels Informed Consent,* Questionnaire and Mindray BS-380. The Data Collection Technique is *Informed Consent* in the form of a consent sheet to be signed by respondents who are willing to be used as research subjects. Questionnaire: a collection of questions used as research support.

Laboratory Inspection, Pre-analysis Prepare the tools and materials used, namely: Mindray BS-380, 500µl micropipette, blue tip, centrifuge, vacutainer tube, syringe, lamp, plaster, alcohol swab, *aluminum foil*, test tube rack and torniquette. The materials used in this study are total bilirubin reagent and serum as examination materials [1]. Analysis: venous blood collection, serum making, sample handling. Based on a referral from Toto Kabila Hospital, the normal value of total bilirubin is <1.1 mg/dL. With a low critical value there is none, while a high critical value is >15 mg/dL.

Data that is not normally distributed will be continued in the Non-parametric analysis test, namely the statistical test of the Kruskal-Wallis *test*. H0 : There was no difference in the results of total bilirubin levels in serum without light exposure that was delayed for 1 hour and 3 hours. Ha : There was a difference in the results of total bilirubin levels in serum without light exposure that was delayed for 1 hour and 3 hours

RESEARCH RESULTS

This scientific work is sent in two stages, namely preparation and implementation. Preparation begins by choosing an initial survey, title, data, research method, and exam proposal.

After obtaining permission from the institution, the research was then sent to the Toto Kabila Hospital in Bone Bolango Regency. The researcher received a research permit from the Gorontalo Province DPMPTSP. The letter was sent to the Director and Training Section of Toto Kabila Hospital after obtaining research permission. After that, the researcher was handed over to the head of the laboratory room to take a blood sample. The total amount of bilirubin is calculated by an automated method. To differentiate the serum, the blood sample is centrifuged for ten minutes at 3000 rpm. Three different treatments were given to the serum: examined immediately, delayed for an hour, and delayed for three hours. This study was conducted for two (two) days, namely from June 30 to July 1, 2024, and the findings produced are as follows:

1. Univariate Data Analysis

1) Characteristics of the Research Subject

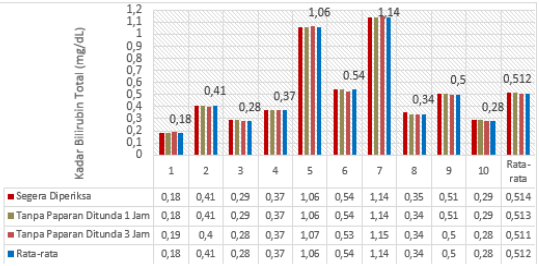
Table 4.1 Characteristics by Age and Gender

	Sum	Average	SD	Min	Mother
Age (years)		21,10	0,994	20	23
Gender (n,%)					
Male	1				
Woman	(10)				
	9				
	(90)				

(*Source: Primary Research Data,* 2024).

Table 4.1 shows the age and gender distribution of respondents. The results showed that 1 male respondent (10%) and 9 female respondents (90%), with an average age of 21.10 and a standard deviation of 0.994 with a minimum age of 20 years and a maximum of 23 years.

2) Results of Observation of Total Bilirubin Levels



	1	2	3	4	5	6	7	8	9	10	Rata-rata
Segera Diperiksa	0,18	0,41	0,29	0,37	1,06	0,54	1,14	0,35	0,51	0,29	0,514
Tanpa Paparan Ditunda 1 Jam	0,18	0,41	0,29	0,37	1,06	0,54	1,14	0,34	0,51	0,29	0,513
Tanpa Paparan Ditunda 3 Jam	0,19	0,4	0,28	0,37	1,07	0,53	1,15	0,34	0,5	0,28	0,511
Rata-rata	0,18	0,41	0,28	0,37	1,06	0,54	1,14	0,34	0,5	0,28	0,512

(*Source: Primary Data of Research*, 2024)

Graph 4.1 Diagram of the Average Examination of Total Bilirubin Levels with three Serum Treatment Groups

Figure 4.1 show the average levels obtained. The bilirubin value in the E(5) code sample was 1.06 mg/dL, while 2 people showed abnormal values (more than 1.1 mg/dL). In the G(7) code sample, the bilirubin value was 1.14 mg/dL. Average value of total bilirubin examination in serum. Meanwhile, the mean value of the total bilirubin examination (immediately checked) with a result value of 0.514 mg/dL was temporarily postponed by 1 hour with an average result of 0.513 mg/dL and serum was postponed by 3 hours with an average result of 0.511 mg/dL

Table 4.2 Average Results of Total Bilirubin Levels in the Three Treatment Groups

Treatment	Up to Bilirubin (mg/dL)		
	Lowest	Highest	Average
No Delay Serum with Light Exposure (Checked immediately)	0.18	1.14	0.514
Serum without light exposure delayed by 1 hour	0.18	1.14	0.513
Serum Without Light Exposure Delayed 3 Hours	0.19	1.15	0.511

(*Source: Primary Research Data,* 2024)

Table 4.2 shows that of the 10 samples studied, the lowest value of total bilirubin examination results with serum without light exposure (Immediately checked) is 0.18 mg/dL, in the 1-hour delayed serum treatment is 0.18 mg/dL, and in the 3-hour delayed serum treatment is 0.19 mg/dL. While the highest value result of the total bilirubin examination with serum without light exposure (Immediately checked) was 1.14 mg/dL, in the serum treatment it was delayed by one hour by 1.14 and in the serum treatment it was delayed by three hours by 0.15 mg/dL.

2. Bivariate Data Analysis

1) Results of Statistical Data Processing

a) Normality Test

Table 4.3 Results of the Normality Test

Normality Test	Shapiro-Wilk		
	Statistic	Df	Sig.
No Delay Serum with Light Exposure (Checked immediately)	.804	10	**.016**
Serum Without Light Exposure is delayed by 1 hour	.803	10	**.016**
Serum No Light Exposure Delayed 3 Hours	.789	10	**.011**

(*Source: Primary Research Data*, 2024)

Based on table 4.3 above, the undelayed serum examination with exposure to lamp light (immediately examined) obtained a significant value of 0.16, the one-hour delayed serum examination was 0.16, and the three-hour delayed serum examination was 0.11, each with a p value <of 0.05. These results show that the data is not normally distributed.

b) Research Hypothesis Test

Table 4.4 Kruskal-Wallis *Test Results*

Test *Kruskal Wallis*	Bilirubin Serum Test Results
Kruskal-Wallis H	.041
Df	2
Asymp. Sig.	**.980**

(*Source: Primary Research Data*, 2024)

Table 4.4 shows the test results of the Kruskal-Wallis test, where from 10 respondents the test result is 0.980 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 above is 0.05 there is no difference.

DISCUSSION

Bilirubin, a yellow pigment, is produced by the overhaul of heme by the reticulonendothelial. After being extracted from the blood, bilirubin is excreted from the body through the enum fluid. [6] found that hyperbilirubinemia is another sign of increased serum total bilirubin levels is a sign of liver dysfunction. Hepatobilia and erythropoietics are activated by the bilirubin test. This is done to diagnose jaundice disease and integrate the development of the disease. The bilirubin test activates hepatobiliary and erythropoietics. Hepatobilier and erythropoietic are activated by bilirubin. To determine and detect diseases, as well as to diagnose jaundice. The bilirubin test activates hepatobiliary and erythropoietics. This test is done to diagnose jaundice disease and integrate the development of the disease. Bilirubin is excreted from the body through the enum fluid after it is extracted from the blood. The bilirubin test activates hepatobiliary and erythropoietics.

[6] found that hyperbilirubinemia is another sign of elevated serum total bilirubin levels is a sign of liver dysfunction. This is done to diagnose jaundice bandages and combine the progression of the disease. The bilirubin test activates the hepatobiliary and erythropoietic systems, which are used to diagnose jaundice disease and integrate the progression of the disease. In order for the examination results

to be accurate, the sample must be treated properly and examined immediately [8]

The study emphasized more on the results of bilirubin levels with experimental sample treatment, so that the samples taken were students by filling out *an informant Consent*, a questionnaire to respondents who had met the criteria for incursion and exclusion that the researcher wanted. The type of experimental research design of *cross-sectional* research with a quantitative approach aims to find a causal relationship between the bound variable (Total Bilirubin Level) and the free variable (treatment group; serum immediately examined, serum delayed 1 hour and serum delayed 3 hours) with numerical results in the form of numbers.

1. Univariate Analysis

The research was carried out by filling out a questionnaire sheet to find the criteria for respondents to be sampled, based on the inclusion and exclusion criteria, respondents were obtained who were ready to be used as research samples with evidence of filling *out informant consent* and questionnaires. Based on the results of interviews with 10 respondents, respondents who stayed up late last night were obtained, respondents were in good health, and respondents who were willing to be the subject of the study. So that in this study the following characteristic results were obtained.

The age and gender of the students who were asked for clinical chemistry examination at the Laboratory of Toto Kabila Hospital, Bone Bolango Regency, are shown in Table 4.1. Nine out of ten people who answered had a venous blood draw. The total bilirubin test value in serum (immediately checked) was average in the three serum treatment groups; The normal test value for 8 people was normal (less than 1.1 mg/dL), and the abnormal test value for 2 people was normal (more than 1.1 mg/dL) on the E and G sample codes, respectively.

Similar results to [5] showed a significant difference between fresh serum and serum that was left for three hours; serum that was left on for three hours had lower total bilirubin levels, with an average of 1,345 mg/dL for fresh serum and 1,081 mg/dL for serum that was left on for three hours.

The results of the decrease in Total Bilirubin levels in some samples occurred in serum without light exposure which was delayed for 3 hours, can be seen in graph 4.1 with sample codes B, C, F, I and J at numbers 2, 3, 6, 9, 10. Temperature and storage time factors are the assumptions of the researcher as the cause of the decrease in the results of Total Bilirubin levels, because the researcher uses laboratory room temperature and delays the examination for 3 hours so that the stability of the serum will be disturbed. While the increase in total bilirubin levels in some samples occurred in no light exposure with a 3-hour delay in examination, see in graph 4.1 with sample codes A,E,G sample numbers 1,5 and 7 in the examination results table [4].

2. Bivariate Analysis

a. Results of the Normality Test Results of Total Bilirubin Levels from the Three Treatment Groups

Based on table 4.3 Data normality test, the normality test results from the three sample treatments (Serum with light exposure without delay, Serum without light exposure delayed for 1 hour, Serum without light exposure postponed for 3 hours) each result from the normality test was (0.016, 0.016 and 0.011). According to the basis for decision-making or interpretation of the normality test results, a p value above 0.05 indicates that the data meets the assumption of normality. Before the evaluation of the results, a non-parametric comparative test is performed. It does not require assumptions about the distribution of population data. When an independent comparison was made between two T-test groups and more than two Anova-test groups, the T-test and Anova-test were both used. The nonparametric analysis was followed by the

Kruskal-Wallis test because this study did not find a normal distribution.

b. Hasil Uji *Kruskal-Wallis* No Parameteric Test

In this study, various factors, including internal and external factors, or the influence of both, affect the stability of the serum. Twelve samples experienced a total decrease in bilirubin levels by 6%, and there was a time effect on immediate bilirubin levels and a three-hour delayed serum. The results of the overlapping sample test produced a significance value of 0.01. The stability of the serum will change during storage because proteins are highly sensitive to physical and chemical changes. Uncontrolled storage time and temperature can alter the initial properties of the serum. The difference occurred because for this study it was carried out with serum samples that were not in closure, in this case the sample was covered with *aluminum foil*, while in the study it was carried out using samples whose storage used *aluminum foil* by reducing or eliminating the effect of the blue light reaction from exposure to direct light either from lamp light or the sun.

In this study, it can be seen that sample codes 2, 3, 6, 8, 9 and 10 there was a decrease in levels of only 0.01 mg/dL in serum treatment without light exposure, 1 hour and 3 hour postponement of examination. This is due to the delay in the examination time and the temperature used is the temperature of the laboratory room. However, for the factors affected by light exposure, the researcher has used a tube cover with *aluminum foil* so that the stored serum is still in a stable state and maintained its stability. So the researchers concluded that temperature and delay were factors in the decrease in the results of total bilirubin levels but still in a normal state.

CONCLUSION

The average value of total bilirubin examination in serum (immediately checked) with an average result value of 0.514 mg/dL, while in serum it was delayed for 1 hour with an average result of 0.513 mg/dL and serum was delayed for 3 hours with an average result of 0.511 mg/dL. The result of the value (significance) > of *alpha* (0.05) or (sig > 0.05) then Ho was accepted and Ha was rejected which means that there was no difference in the results of total bilirubin levels in serum without light exposure which was delayed by 1 hour and 3 hours.

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