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Exploring the Relationship between HIV Rapid Testing and HIV Viral Load in HIV Patients at Haji Hospital, Surabaya, Indonesia

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ABSTRACT HIV (Human Immunodeficiency Virus) is a virus that causes decreased immunity. Examinations that can be done for HIV testing include a rapid HIV test and HIV viral load. The rapid HIV test is a test that is used to find out the antibodies in the sample, while the viral load test is to find out the amount of HIV. The purpose of this study was to compare HIV using a rapid test with HIV viral load in people with HIV. This type of research is observational with a cross-sectional approach. The sample in this study were 30 samples from HIV sufferers who carried out an HIV viral load examination using the Molecular Rapid Test (TCM) method at the RSUD Haji Provinsi Jawa Timur, then a rapid HIV test was carried out using the immunochromatography method. The results of the HIV rapid test and HIV viral load were processed in the form of a bar chart and tested with the McNemar statistical test. The results of the McNemar test obtained Exact Sig. (2-tailed) is 0.000, which means that the significance value (p) is smaller than the α value (0.005), so it can be concluded that there is a significant difference between the results of the HIV viral load test and the HIV rapid test. The results of this study indicate that HIV sufferers with undetectable HIV viral load results indicate that the patient is not completely free of the virus.

INDEX TERMS HIV, rapid test, viral load HIV.

I. INTRODUCTION

HIV (Human Immunodeficiency Virus) is a virus that causes decreased immunity. This virus attacks and damages parts of the immune system that play an important role in preventing infection. T lymphocyte cells or helper T cells are cells that function in preventing infection in the body. Disruption of the immune system can result in opportunistic infections (OI). Opportunistic infections cause a variety of clinical symptoms for HIV sufferers, this indicates that the patient is at the AIDS stage[1]. Since 1981 until now, AIDS (Acquired Immune Deficiency Syndrome) and HIV (Human Immunodeficiency Virus) have become health problems in every country because until now infection from HIV cannot be cured. This aims to extend the life span of HIV sufferers like non-HIV people in general [2].

In 2021, the United Nations Program on HIV and AIDS (UNAIDS) recorded 38.4 million cases of HIV infection in the world and 650,000 people died due to AIDS. Meanwhile, in Indonesia, the development of HIV AIDS in January - March 2021 recorded 7,650 people living with HIV AIDS (PLWHA).

So that as many as 427,201 HIV cases of PLHIV and 131,417 AIDS cases have been reported cumulatively until March 202[3].

One of the prevention efforts is the early detection of HIV infection which requires laboratory tests. Serological tests are laboratory procedures used to determine the diagnosis of HIV. In addition, there is a viral load test that serves as a marker to monitor the response to ARV therapy by measuring the amount of HIV in the sample[4].

The rapid test is a serological examination that functions to qualitatively detect HIV antibodies in a sample. This inspection has an easy work procedure and does not require special tools so that it can be carried out in the field. However, the results of serological examinations in HIV are greatly influenced by the sensitivity and specificity of the device used [5]. The results of the rapid test did not show the development of HIV in the body, so there is a need for other supporting tests, one of which is a viral load test.

Viral load examination is a quantitative examination to determine the amount of virus in the sample. There are various

methods for checking viral load. The gold standard of this examination is the polymerase chain reaction (PCR) method. However, research conducted by [6] shows that the rapid molecular test (TCM) method is better than the PCR method with the HIV/AIDS program.

Based on research conducted by [7], HIV viral load testing can show results that are not detected in people with HIV. Meanwhile, from research that has been conducted by [8], it was found that the rapid test had a positive predictive value and a negative predictive value of 100% so the examination obtained results by reality by 100%. However, according to [9], the addition of a second rapid test to confirm a positive first rapid test result has a positive predictive value of 100% compared to a single rapid test. Based on these problems, research was conducted on the relationship between the results of the HIV rapid test and the results of the HIV viral load using the Molecular Rapid Test (TCM) method. The purpose of this study was to analyze the comparison of HIV using a rapid test with HIV viral load in HIV patients at Haji Hospital in Surabaya.

II. METHODOLOGY

In this study, EDTA plasma samples were used. This is because the use of specimens other than samples with EDTA and citric acid dextrose (ACD) causes inaccurate results [10]. The examination was carried out from February to March 2023 at the Haji Hospital Laboratory in East Java Province.

A. SAMPLE PREPARATION WORK STEPS

Centrifuge the EDTA blood specimen to separate plasma from other blood components at 3000 rpm for 10 minutes. After that, transfer the plasma to the sample tube that has been prepared. Then give identity. The sample can already be examined. If not checked immediately, it can be stored at -20°C. The sample in the study was taken by using a quota sampling technique so that the number of samples obtained was 30 respondents. The inclusion criteria were patients who had their HIV viral load checked at the Haji Hospital in Surabaya.

B. HIV RAPID TEST METHOD

A person infected with HIV will have antibodies in the body that will bind to the HIV recombinant antigen on the HIV cassette test. HIV-1 recombinant antigens are gp120, gp41, and p24, while HIV-2 is gp36. The rapid test method used uses the principle of immunochromatography, namely the presence of antibody-antigen bonds will form a red line in the control zone and the test zone [11].

C. WORK STEPS IN AN HIV RAPID TEST EXAMINATION

Drop 10 µl of serum or plasma or 20 µl of whole blood into the specimen well. Then, add 3 drops of diluent buffer into the specimen well. Wait about 15-20 minutes to see the results. The interpretation of the results of this examination is that the appearance of a line in test areas 1 and/or 2 and control (C)

shows a positive result. Meanwhile, the line that only appears in the control (C) shows a negative result. If no line appears in the control area (C), the result is invalid, it is necessary to re-examine.

D. HIV VIRAL LOAD METHOD

The method for testing HIV viral load is the Molecular Rapid Test (TCM) with a working principle such as Real Time Polymerase Chain Reaction (RT-PCR). The HIV viral load test detects the amount of HIV in the sample. The results obtained are in the form of RNA copies/ml.

E. WORK STEPS IN HIV VIRAL LOAD TESTING

Prepare samples that have been prepared beforehand. If the sample is frozen, it needs to be allowed to stand until it reaches room temperature. Then vortex for 15 minutes so that the sample is homogeneous. After that, prepare the Xpert HIV-1 VL cartridge and give the sample identity to the cartridge. Then pipette at least 1 ml of plasma sample and put it into the cartridge. After processing the cartridge on the GeneXpert machine [12].

III. RESULTS

In the results of the viral load examination using the GeneXpert tool, there is a number indicating the amount of virus in the form of copies per mL (copies/mL), which indicates that the viral load has been detected. TABLE 1 and TABLE 2 present the results of HIV viral load tests.

TABLE 1
Results of viral load examination by age group

No	Age group	Identity code	Viral Load (Copies/ml / Not Detected)
1	20-24 years old	114L	187
		119L	<40
2	25-49 years old	101P	Not Detected
		102P	Not Detected
		103L	<40
		104L	71
		105L	59200
		106L	Not Detected
		107L	<40
		108L	Not Detected
		109L	Not Detected
		110L	<40
		111L	<40
		113P	Not Detected
115L	<40		
116L	Not Detected		
117L	<40		
118P	<40		
120L	<40		
121L	<40		
122L	356000		

No	Age group	Identity code	Viral Load (Copies/ml / Not Detected)
		124L	Not Detected
		125L	Not Detected
		126L	Not Detected
		127L	Not Detected
		128L	Not Detected
		129L	Not Detected
		130P	Not Detected
3	>50 years old	112P	Not Detected
		123L	303000

TABLE 2
Results of viral load examination by gender

No	Gender	Identity Code	Viral Load (Copies/ml / Not Detected)
1	Man	103L	<40
		104L	71
		105L	59200
		106L	Not Detected
		107L	<40
		108L	Not Detected
		109L	Not Detected
		110L	<40
		111L	<40
		114L	187
		115L	<40
		116L	Not Detected
		117L	<40
		119L	<40
		120L	<40
		121L	<40
		122L	356000
		123L	303000
		124L	Not Detected
		125L	Not Detected
		126L	Not Detected
		127L	Not Detected
		128L	Not Detected
		129L	Not Detected
2	Woman	101P	Not Detected
		102P	Not Detected
		112P	Not Detected
		113P	Not Detected
		118P	<40
		130P	Not Detected

Based on TABLE 1, the results of the HIV viral load examination showed that the lowest value in the 20-24 year age group was <40 copies/mL, and the highest value was 187 copies/mL. In the age group of 25-49 years, the lowest viral load was not detected and the highest was 356000 copies/mL. Meanwhile, in the age group >50 years, the lowest viral load was undetectable and the highest viral load was 303000 copies/mL. TABLE 2 shows that the lowest value of the HIV

viral load test results in men was undetectable and the highest value was 356000 copies/mL, while in women the lowest viral load value was undetectable and the highest value was <40 copies/mL.

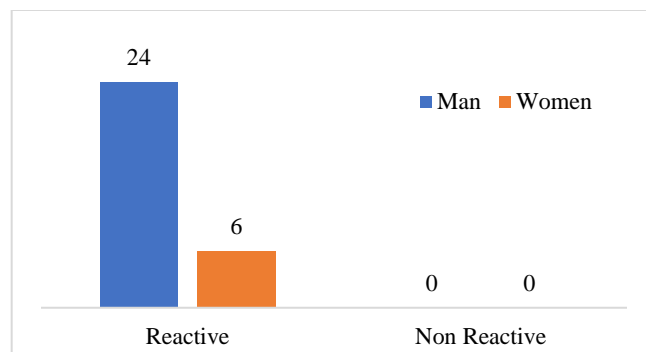


FIGURE 1. Number of rapid test results based on gender

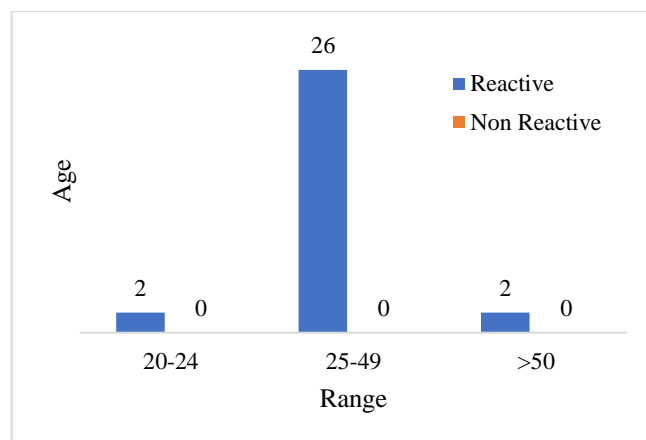


FIGURE 2. Number of rapid test results by age group

FIGURE 1 shows that the highest percentage of reactive results is in the male sex. Furthermore, based on gender, FIGURE 2 shows that the age group 25-49 years has the highest percentage of giving reactive results on the rapid test.

TABLE 3
Cross-tabulation of HIV rapid test and viral load results

Viral Load	Rapid Test	
	Reactive	Non reactive
Detected	15	0
Not Detected	15	0
Total	30	

TABLE 3 shows the results of the viral load examination, both detected and undetected, getting reactive results on the rapid test. In the table, there are the same number, namely 15 patients (50%), with reactive results on the rapid test. TABLE 4 shows.

IV. DATA ANALYSIS

The results of statistical tests using the exact sig value. McNemar's test is $0.000 < \alpha = 0.005$. So it can be concluded that there is a significant difference between the results of the viral load examination and the results of the HIV rapid test.

V. DISCUSSION

This study was conducted to compare the results of HIV testing using a rapid test with HIV viral load. Plasma samples from HIV/AIDS sufferers were first examined for viral load using the GeneXpert tool. At this stage of the examination, the first check is carried out on the plasma sample. The plasma that is good for viral load testing is clear yellow in color and has no clots, lysis, or lipemia because these things can interfere with the testing process. According to [13] the presence of blood cells and components in the sample, such as hemoglobin and lactoferrin, can inhibit DNA polymerase and cause low amplification efficiency, inaccurate quantification, and even amplification failure. The sample used for viral load testing has met the requirements for testing. The sample used for viral load testing has met the requirements for testing. As much as 1 mL of plasma sample was put into the provided cartridge, then the cartridge was processed using the GeneXpert tool.

After getting the results of the viral load examination, the remaining plasma samples were used for a rapid test using the immunochromatography method. Pipette the plasma using the pipette provided in the test kit, as much as 1 drop or about 30 μ L of plasma is dropped on the test cassette, then 1 drop of buffer is added.

Based on research results, the highest percentage of HIV sufferers occurs in the age group of 25-49 years, which is the productive age group. This is in line with research by [14] which shows that a higher risk occurs in the age group of 30 to 40 years. This age group is a productive period in which many individuals explore, form self-identities, and determine the desired lifestyle. So the influence of the unfavorable environment in an association can contribute to the occurrence of sexual behavior which can lead to high HIV incidence rates. Meanwhile, based on gender, HIV sufferers are mostly male than female. These results are in accordance with data from the Indonesian Ministry of Health for 2022 which recorded that HIV sufferers in January - September 2022 were more common in men (70.33%) than women (29.67%). The high incidence rate in males compared to females can be caused by the behavior and patterns of association in males. This behavior and association allows them to interact with many individuals in various environments who may be exposed to a high risk of HIV infection.

TABLE 3 shows a total of 15 samples with detectable viral loads and reactive results on the rapid test. However, 15 samples showed reactive results on the rapid test but were undetectable on the HIV viral load examination.

Reactive results on the rapid test occur because there is a virus that is still active in the patient's body so that the immune response continues to form to fight HIV infection. The formation of antibodies against HIV is a sign that the immune

system is fighting the virus. According to [15], activated CD4 lymphocytes by releasing cytokines will cause the formation of antibodies. The presence of antibodies in the plasma will bind to the recombinant antigen found in the rapid test kit so that a colored line forms in the test zone on the tool.

An undetectable result on an HIV viral load test can occur due to a small amount of virus in the blood. This is in line with [16], which states that undetectable viral load results occur because the number of copies/mL detected is below the detection limit or the HIV-1 Ct value is not obtained so results are reported as undetectable.

There are reactive results on the rapid test and the results of the viral load examination are not detected because there are antibodies in the body, but the amount of virus in the body is small. This is due to the body's specific immune response to HIV being able to control the virus so that the amount of virus in the blood decreases. According to Sudigdoadi (2015) cells infected with HIV will be lysed by CD8+ cytotoxic T cells, and cytokines from CD8+ will inhibit viral replication in CD4+ cells without lysing cells resulting in a decrease in the amount of free virus and virus present in cells. In addition, antiretroviral therapy (ARV) also affects suppressing viral replication in the body[17]. ARVs work by inhibiting the reproduction of HIV in the body, thereby helping to control the infection. This can result in low or undetectable HIV viral load.

In processing the statistical test data (TABLE 4), results were found which showed a significant difference between the results of the HIV viral load examination and the HIV rapid test. This difference occurred in this study because the objectives of the two types of examination were different. Viral load (VL) examination is a test to determine the amount of HIV present in each milliliter of blood expressed in units of 'copies/mL'. In addition, VL examination can measure the magnitude of viral replication and has a role in the course of HIV infection[4]. Meanwhile, the HIV rapid test is one of the examination methods used to detect the presence of antibodies in plasma or serum blood samples.

HIV antibodies in the sample generally form after at least 3 months of infection. Therefore, identification of HIV antibodies should be done at least 3 months after the infection occurred. This is due to the existence of a window period in HIV [18]. One of the examination methods used to detect HIV antibodies is a rapid test. This rapid test method has been evaluated by a national reference laboratory using specimens from Indonesia, which provides a sensitivity and specificity value that represents the performance of the rapid test in examining Indonesian specimens [19]. While the amount of virus in the blood can be done after the individual is infected or in the window phase (window period). According to Pitchumoni & Brun (2022), viremia will develop more than 15 days after infection [20].

All samples studied showed reactive results on the HIV rapid test. However, the HIV viral load test yielded detected and not detected results. So it should be noted that the virus is

not detected on the HIV viral load examination so it cannot guarantee that there is no risk of transmission. According to research by DeHaan et al. (2022), individuals who have low or undetectable viral loads have a low risk of HIV transmission [21].

Low or undetectable viral load results in people with HIV are common in patients who are on routine ARV therapy. Compliant use of ARVs can suppress viral replication, thereby improving the quality of life of people with HIV [22]. In a study conducted by Putri et al. (2022), it was found that patients who were influenced by drug use with discipline were able to achieve viral loads below 10,000 copies/ml, and some patients did not even have detectable virus levels in the body [23]. This is in line with [24] the amount of undetectable HIV-1 virus (<40 copies/mL) as the main marker that sufferers have good adherence in using ARV drugs.

From the two tests, it can be concluded that the HIV viral load test is a better test than the rapid test. However, HIV diagnosis can be made using an HIV Rapid test by implementing a rapid test testing strategy in HIV diagnosis. This is done because the limited number of targeted antigens causes an increased risk of false positive results so the implementation of this testing strategy can maximize the accuracy of the test results and minimize costs [25].

The limitations of this study are respondents with age criteria of 15 to 65 years. In addition, the weakness of this study did not pay attention to the length of ARV treatment that patients received. The implication of this study is to add to scientific information regarding HIV testing using the rapid test and HIV viral load using the Molecular Rapid Test method.

VI. CONCLUSION

The purpose of this study was to analyze the comparison of HIV using a rapid test with HIV viral load in people with HIV/AIDS. In this study, it can be concluded that there is a significant difference between the results of the HIV rapid test and the results of the HIV viral load examination. The results of the rapid test showed that 30 samples (100%) were reactive, while the results of the HIV viral load examination showed that 15 samples (50%) were detected and 15 samples (50%) were not detected. For future researchers, it is hoped that they can conduct further research using a larger number of samples and pay attention to the length of time patients receive ARV therapy.

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