Comparisson Of The Examination Of Mycobacterium Tuberculosis (M.Tb) Using Polymerase Chain Reaction (PCR) And ZiehlNeelsen (ZN) On Tuberculosis (Tb) Patients Suspect In Public Health Facilities Of Manado

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ABSTRACT

(TB) is an infectious disease caused by Mycobacterium tuberculosis. TB infections occur through the air, namely the inhalation of droplets containing germs from an infected person. Four People exposed with Mycobacterium tuberculosis have a 10% risk of developing pulmonary TB. The aim of this research was to compare of the examination of M.Tb using polymerase chain reaction and ziehlneelsen on TB Patients suspect in Public Health Center of Manado. The research method used was analytic laboratory examination. As the result, TB examination using ZiehlNeelsen method found 13 samples with positive TB, while using the PCR examination method 93 samples (93,6%) showed positive. There was significantly differences between PCR and ZiehlNeelsen examination method. TB examination using the PCR method had high sensitivity and specificity to detect Mycobacterium tuberculosis, so that more MTB bacteria can be detected than using the ZN test. It is recommended that health workers at the Public Health center to diagnose TB should use an examination that has high sensitivity and specificity so that the results obtained are more accurate.

Key Word: Mycobacterium Tuberculosis, ZiehlNeelsen method Polymerase Chain Reaction Method

BACKGROUND

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. These rod-shaped and aerobic germs are acid-resistant. The majority of TB infections occur through the air, namely the inhalation of droplets containing germs from an infected person. Four people infected with Mycobacterium tuberculosis have a 10% risk of developing pulmonary TB. People with compromised immune systems, such as people living with HIV, diabetes, malnutrition, diabetes or people who use tobacco, are at higher risk of getting sick.(Ramadhan et al., 2017)

Data from the World Health Organization or the World Health Organization (WHO) states that as many as 10.4 million people in the world are infected with TB. Of these, 1.7 million people died and 0.4 million of them also suffered from HIV. TB infection has resulted in more than 95% of deaths in low- and middle-income countries. TB disease is the first cause of death caused by infectious diseases and is one of the ten deadliest diseases in the world. Several

countries that contributed to 64% of new TB cases were India, Indonesia, China, Philippines, Pakistan, Nigeria, and South Africa. Indonesia is in third place out of six countries that account for 60% of TB cases worldwide, after China and India. (Khariri, 2020)

The highest pulmonary tuberculosis sufferers are in the productive age, namely the age of 15-50 years, around 75%. A person affected by tuberculosis, especially an adult, is estimated to lose an average of 3-4 months of work time which can result in a loss of about 20-30% of his household income. Meanwhile, someone who dies from tuberculosis will lose his income for about 15 years. In addition to making economic losses, tuberculosis also has other bad effects, namely being ostracized from the community (WHO, 2013). One of the indicators needed in controlling pulmonary TB is the Case Notification Rate (CNR), which is a number that shows the number of new patients found and recorded among 100,000 residents in a certain area. The CNR indicator is useful in showing an increasing or decreasing trend in the discovery of new patients in the area. (Arivany, 2017)In ensuring the diagnosis of pulmonary TB, the minimal examination that needs to be done is a microscopic examination of AFB. This examination plays a role in early diagnosis or monitoring of pulmonary TB treatment. The method used in this examination is the ZiehlNeelsen method. This method is the method recommended by WHO. The sample used is sputum. Purulent sputum will be made into preparations, then after drying it is added with CarbolFuchsin and heated until it evaporates. Heating and adding CarbolFuchsin aims to open the M.TB cell wall which contains a layer of fat in high concentrations so as to facilitate the absorption of the dye. This will also make the dye insoluble by even strong bleaches such as alcoholic acids. There are shortcomings in this method, namely in the manufacture of preparations using sputum containing live M.TB. This can cause transmission of these bacteria to laboratory workers who work on it (Romi, 2020)

Detection of Mycobacterium tuberculosis in sputum can be done by means of polymerase chain reaction (PCR), microscopic examination, and bacterial culture. Five Microscopic examination of sputum is a key component in the TB control program to establish a diagnosis, evaluation and follow-up treatment from examination of 3 sputum specimens in the morning (SP). Microscopic examination of sputum is the easiest, cheapest, efficient, specific and can be carried out in all laboratory units. Five Detection of TB germs by PCR technique has a very high sensitivity. PCR is a way of amplifying DNA, in this case Mycobacterium tuberculosis DNA, in vitro. This process requires a double-stranded DNA template containing the target DNA, DNA polymerase enzyme, nucleotide triphosphate, and a pair of primers. (Ramadhan et al., 2017) The aim this research was to compare   of the examination of M.Tb using polymerase chain reaction and ziehlneelsen on TB Patients suspect in Public Health Center of Manado.

METHOD OF RESEARCH

Research type is analytic laboratory examination on TB patients suspect had have been cough with mucus as long a 2 weeks, bleeding cough, fever, diaphoresis, loss body weight, anorexia, dispnoe, fatigue, with population as much as 120 patients. Samples taken by Slovin formula as amount 99 respondents was met inclusion criteria. Sample sputum taken by home respondent with home visit. Data sample collecting with method sputum was collected into the container and then carrying to Medicine Technology laboratory of Manado Health Polytechnic to be done examination using PCR and ZiehlNeelsenmethod. Asking respondent to giving informed consent and signed it. Examination step followed pra analytic, analytic, and pascaanalytic. After

examination, then result of examination to be done statistic analyze using Wilcoxon and then presented by frequent distribution tables with explanation and discussion.

RESULTS AND DISCUSSION

Table 1. Distribution of Age, Sex, Education, Occupation, Detection Results of M.TB Using PCR and ZiehlNeelsen Methods On TB Patients Suspect in Manado

No	Demografi Data, Results of PCR and ZiehlNeelsen	f	%
1.	Aged:		
	a. 12-25 years	12	12,1
	b. 26-64 years	80	80,8
	c. >65 years	7	7,1
	Sex:		
2.	a. Male	49	49,5
	b. Female	50	50,5
3.	Education:	15	15,2
	a. Elementary School	19	19,2
	b. Secondary School	57	57,6
	c. High School	8	8,1
	d. University		
	Occupation:		
4.	a. House Hold	35	35,4
	b. Student	5	5,1
	c. Particular	26	26,3
	d. Labourer	13	13,1
	e. Other worker	20	20,2
	PCR Examination Results:		
5.	a. Detected	93	93,6
	b. No Detected	6	6,3
	ZiehlNeelsen Examination Results:		
6.	a. Positive	13	13,1
	b. Negative	86	86,9
	Total respondents = 99		

Test Statistics[a]

	ZiehlNeelsen- Polymere Chain Reaction
Z	-8.721[b]
Asymp. Sig. (2-tailed)	.000

a.   Wilcoxon Signed Ranks
b.   Based on negative ranks

Based on research result showing that most of respondents age productive is 26-64 years as amount 80 (80,8%), sex almost same with female and male as follow 50(50,5%), and 49(49,5%), education level is high school as much as 57(57,6%), occupation is household mother is

35(35,4%). Ziehlneelsen examination result is negative 86(86,9%), PCR examination result detected is 93(93,6%),. Based on statistical analyze with Wilcoxon test showing that p0,000. It mean there is significantly differences comparison between ZiehlNeelsen with PCR examination method. Where PCR examination better more than ZiehlNeelsen method.

DISCUSSION

The research results of sputum examination of suspected pulmonary tuberculosis by gender, based on research conducted by Sulistyawai and Ramadhan, (2021). Even based on the Tuberculosis Prevalence Survey, the prevalence in men is 3 times higher than in women. The same is happening in other countries. This may be because men are more exposed to TB risk factors, such as smoking and lack of medication adherence. This survey found that of all male participants who smoked as much as 68.5% and only 3.7% of female participants who smoked. Other hand, the results showed that the characteristics of TB patients were mostly in the groups: productive age aged 12-35 years and adult age range 49-61 years; male gender; elementary school education; laborer's work; low socioeconomic status; active smoker. It was found that 13.2% of TB patients with DM, 45% of TB patients with hypertension, 47.5% of TB patients with anemia, 55% of TB patients with malnutrition, 17.5% of patients had a family history of TB. Patients relapsed in 12.5%; patients who dropped out of OAT 17.5%; and was found not to take OAT as much as 15%.

The research results of sputum examination according to age, that is, based on research conducted by Sharma, pulmonary TB is more common in the productive age group (15-50 years), low economic status and lack of education (Sharma, 2017). Similarly, research conducted by (North et al., 2022) said that the age of pulmonary TB disease was most often found in the productive age, namely 15-50 years. nowadays with the demographic transition, causing the life expectancy of the elderly to be higher. At the age of more than 55 years a person's immunological system decreases, so that he is very susceptible to various diseases, including pulmonary TB. In addition, productive age is easier to become a source of infection because of its mobility. This is also the same as research conducted by Sulistyawati and Ramadhan (2021) who said that productive age is the age where a person is at the stage of working/producing something both for oneself and for others. 75% of patients with pulmonary TB are found in the most economically productive age (15-49 years), at that age if a person suffers from pulmonary TB, it can result in the individual being unproductive and even a burden to his family (Sulistyawati and Ramadhan 2021). Characteristics respondents tb male is age between 45 to 54 years, 55 to 64 years and education did not finish primary school, graduate high school, and work as laborer / farmer. The government is continuing to intensify health education activities for community about how to prevent of TB , to finding TB patients and perform intensive treatment until healed (Fitria, et al, 2017).

The result demonstrated there were difference with research by Kanchanasuwan, and Kositpantawong, (2021), Research result with samples from 92 patients with scanty AFB smear were processed for RT-PCR. There were 26 (28.3%) isolates having positive RT-PCR test results. Of these 26 isolates that RT-PCR positive, 25 (96.2%) were culture positive, while only 1 (3.8%) were culture negative. When compared to standard culture, sensitivity, specificity, and positive and negative predictive values of the GeneXpert system for respiratory samples were 100%, 98.7%, 87%, and 100%, respectively; these values for nonrespiratory samples were 71%, 98.6%, 71%, and 98.6%, respectively. For this purpose, easy to-use new methods that can

provide reliable and fast results with high specificity and sensitivity are being sought. Early diagnosis is of great importance for the treatment of tuberculosis that the GeneXpert MTB/RIF is a rapid and reliable system that can be employed in the diagnosis of tuberculosis, and when utilized together with conventional tests, it can make significant contributions to tuberculosis diagnosis, Bilqin, et,al (2016). The result of BTA staining from Bojonggede Public Health Center was 84 samples, BTA positive was 35 samples (42%), BTA negative 49(58%). PCR test result from 20 samples was 14 positive BTA (70%), negative is 6 samples (30%) that's mean people who do not suffer from tuberculosis. The conclusion of this research is PCR test result compared with result of BTA test with ZiehlNeelsen, that is the result of positive test of the percentage is bigger than semar staining test result, Ikhsan, et.al, (2017). PCR can be useful compared to smear microscopy and culture methods and is applicable as a rapid screening test for child TB. A larger scale study is required to determine its diagnostic efficacy in improving the detection of child TB in the presence and absence of severe malnutrition, (Kabir, et al, 2017).

CONCLUSION AND RECOMMENDATION
Tuberculosis diagnosis using PCR method was found to be more effective, sensitive, and specific than ZiehlNeelsenmethod.This research "also" found that M.TB Scanty after continuous of examination using PCR method, was detected. This Research recommendation that as well using PCR method to conducted early diagnostic on TB Patients suspect when conventional method result is scanty.

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