DESCRIPTION OF SPUTUM EXAMINATION WITH ZIEHL-NEELSEN AND GENEXPERT METHOD IN TUBERCULOSIS SUSPECT PATIENTS AT KABILA PUSKESMAS 2020

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ABSTRACT

Tuberculosis is a chronic infectious disease caused by the group of bacteria Mycobacterium tuberculosis which usually attacks the lung organs and then can attack all parts of the body and can cause a disease known as TB disease. TB examination can be done with several techniques, namely microscopic examination with Zhiel-Neelsen staining to detect Acid-Resistant Bacteria (BTA) and GeneXpert examination as a Molecular Rapid Test (TCM) to diagnose Drug-Resistant TB (TB RO).

The purpose of this study was to describe the results of sputum examination using the Zhiel-Neelsen and GeneXpert methods in suspected tuberculosis patients at Kabila Health Center. Quantitative research methods with a descriptive approach. The population in this study were all suspected patients who were examined in the Kabila Public Health Center laboratory with a total sample of 23 people, microscope instruments and GeneXpert with univariate data analysis. The results obtained showed more negative results, namely 12 of 23 examination samples (52. 2%) with GeneXpert results showing 11 MTB samples not detected from 23 samples (47.8%).

From the results shown, it can be seen that the picture of the different examination results from these two examinations is that there are samples with negative results on the Zhiel-Neelsen method and tested positive (MTB Detected Low) with the GeneXpert examination. We recommend that the sample be referred to a place where there is a GeneXpert health facility so that the patient can be diagnosed more quickly so that the spread of TB disease is not wider and treatment can be given immediately.

Keywords:tuberculosis, zhiel-neelsen, gene-xpert

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by the Mycobacterium tuberculosis group of bacteria which usually attacks the lung organs and then can attack all parts of the body and can cause a disease known as TB disease[1]. It is usually found in the upper lung lobe where the air flow is quite good[2]. These bacteria have acid-resistant properties so they are called acid-resistant bacteria (BTA) and are types of bacteria that can not be stained by ordinary aniline staining except by using phenol and by heating[3].

Global Tuberculosis Report(2017) reported that there were new TB cases which reached 10.4 million cases, which is equivalent to 120 cases per 100,000 populations. The five countries with the highest incidence of cases are India, Indonesia, China, the Philippines and Pakistan. Most of the estimated incidence of TB in 2016 occurred in Southeast Asia,

amounting to 45%, where Indonesia is one of them and 25% occurred in the African region, as much as 3% in America, as much as 3% in Europe, as much as 7% in the East Mediterranean, and West Pacific as much as 17%[4].

The number of TB cases reported by the Gorontalo Provincial Health Office in 2019 saw the discovery of new TB cases in districts and cities, namely in Gorontalo District there were 1417 cases, Gorontalo City had 732 cases, Bonebolango Regency had 639 cases, Pohuwato Regency 447 cases, North Gorontalo District had 342 cases, and in Boalemo District there are 338 TB cases. So that TB disease is also a problem of high concern because it is an infectious disease[5].

TB is still a big problem for the government and society, especially in developing countries like Indonesia because it still has weaknesses in effective detection methods. TB disease is a deadly health threat. This contributes to the worldwide problem of TB, because TB patients who do not receive proper treatment can become a source of infection in the community and even die[6].

TB examination can now be done with several techniques, including microscopic examination with acid-resistant staining to detect Acid-Resistant Bacteria (AFB) and GeneXpert examination as a Molecular Rapid Test (TCM) to diagnose TB and diagnose Drug-Resistant TB (TB RO).There are several acid-resistant staining techniques for staining these bacteria.One of the direct examination of microscopic sputum smears is the Zhiel-Neelsen stain type[7] with the principle of Zhiel-Nelseen staining, namely M. tuberculosis has a lipid wall layer (mycoladic acid) which is resistant to acid with the heating process makes it easier to enter carbol fuchsin even though it is decolarized with alcoholic acid, however this method has low sensitivity, is unable to determine drug sensitivity, and has different qualities because it is influenced by the skill level of the technician in conducting the examination[8].

Efforts to enforce the initial diagnosis of TB in patients can not only be done by using Zhiel-Neelesen staining but also other techniques, namely by using TCM with the Genexpert tool with the principle of examination, namely the molecular detection method based on nested real-time PCR for TB diagnosis. The PCR primers used were able to amplify approximately 81 bp of the core region of the MTB rpoB gene complex, whereas the probe was designed to distinguish wild type sequences and mutations in the core region associated with rifampin resistance[8]. GeneXpert is a test tool that uses a cartridge based on the Nucleic Acid Amplification Test (NAAT) automatically to detect TB cases and diagnose TB RO quickly and accurately and can be done even if the sputum sample is only 1 ml,required for the PCR (Polymerase Chain Reaction) process in one cartridge[8].

RESEARCH METHODOLOGY

This research uses quantitative research with a descriptive approach to explain the results of the research obtained. This research was conducted from 31 August - 28 September 2020, the research location was located at the Kabila Health Center Laboratory, Bone Bolango Regency. The tools and materials used during the examination were sample pots, slide objects, microscopes, GeneXpert, Bunsen lamps, disposable Genepert pipettes and catheters as well as Zhiel-Neelsen reangents, oil immersion, buffer reagents and sputum samples. Collecting data using accidental sampling technique with a total sample of 23 patients with suspected tuberculosis who carried out sputum examinations at the Kabila Health

Center Laboratory using a sample size determination formula with an estimate of the proportion of the approved sample population. Data were analyzed using univariate analysis and processed with Statistical Package for Social Science (SPSS). The sample size obtained is based on the following formula:

$$n = \frac{N.\ Z^2 1-_{\alpha/2}\ P\ (1-P)}{(N-1)\ d^2 + Z^2 1-_{\alpha/2}\ P\ (1-P)}$$

$$n = \frac{86.\ 1,64^2.\ 0.5\ (1-0.5)}{(86-1)\ 0.15^2 + 1,64^2.\ 0.5\ (1-0.5)}$$

$$n = \frac{86.\ 2,6896.\ 0.5\ (0.5)}{(85)\ 0.0225 + 2.6896.\ 0.5\ (0.25)}$$

$$n = \frac{57,8264}{2,5849}$$

n = 22.37 = 23 samples

So the sample size used as a study of 23 samples.

Information :
N = Total Population
n = Number of samples
$Z^2 1-_{\alpha/2}$ = Degree of significance, usually 90% =1.64
P = The proportion of a particular case to the population, based on previous research known the proportion, is set at 50% = 0.5

RESEARCH FINDINGS

Observation of the description of the results of sputum examination using the Zhiel-Neelsen and GeneXpert methods in TB suspected patients at Kabila Health Center with the total number of samples examined as many as 23 sputum samples carried out 2 examinations on the same patient sample using the Zhiel-Neelsen and GeneXpert staining method obtained results such as shown in the table below:

Table 1.

The results of the Zhiel-Neelsen staining examination

Zhiel-Neelsen Method Examination		
Result	Frequency	Percentage (%)
Negative	12	52.2
Scanty	0	0
Positive 1	4	17.4
Positive 2	6	26.1
Positive 3	1	4.3

Source: Data Processed (2020)

Based on table 1, it can be seen that the results of the examination with the Zhiel-Neelsen method are negative results as much as 12 (52.2%), Scanty as many as 0 (0.0%), positive 1 by 4(17.4%), positive 2 was 6 (26.1%) and positive 3 was 1 (4.3%).

Tabel 2.

Examination Results of the GeneXpert Method

GeneXpert Method Examination		
Result	Frequency	Percentage (%)
MTB Not Detected	11	47.8
MTB Detected Very Low	0	0
MTB Detected Low	3	13.0
MTB Detected Medium	8	34.8
MTB Detected High	1	4.3

Source: Data processed (2020)

Based on table 2, it can be seen that the results of the examination with the GeneXpert method are 11 (47.8%) MTB Not Detected, 0 (0.0%) Detected Very Low, 3 (13.0%) MTB Detected Low, 8 (13.0%) MTB Detected Medium (34.8%) and MTB Detected High as much as 1 (4.3%).

Table 3.

Summary of examination results of the Zhiel-Neelsen and GeneXpert methods

Zhiel-Neelsen	Frequency	Percent (%)	GeneXpert	Frequency	Percent (%)
Negative	12	52.2	MTB Not Detected	11	47.8
Scanty	0	0.0	MTB Detected Very Low	0	0.0
Positive 1	4	17.4	MTB Detected Low	3	13.0
Positive 2	6	26.1	MTB Detected Medium	8	34.8
Positive 3	1	4.3	MTB Detected High	1	4.3
Total	23	100%	Total	23	100%

Based on table 3 it can be seen that the results of the microscopic examination of Zhiel-Neelsen staining with negative categories were 12 (52.2%), scanty categories were 0 (0.0%), positive category 1 was 4 (17.4%) positive category 2 were 6 (26.1%) and positive category 3 as much as 1 (4.3%). Then the results of the GeneXpert examination which were identified as MTB Not Detected were 11 (47.8%), the results identified as MTB Detected Very Low were 0 (0.0%), the results identified as MTB Detected Low were 3 (13.0%), the results identified as MTB Detected Medium was 8 (34.8%) and the results identified were MTB Detected High as much as 1 (4.3%).

Picture 1.

Graph of Zhiel-Neelsen and GeneXpert examination results
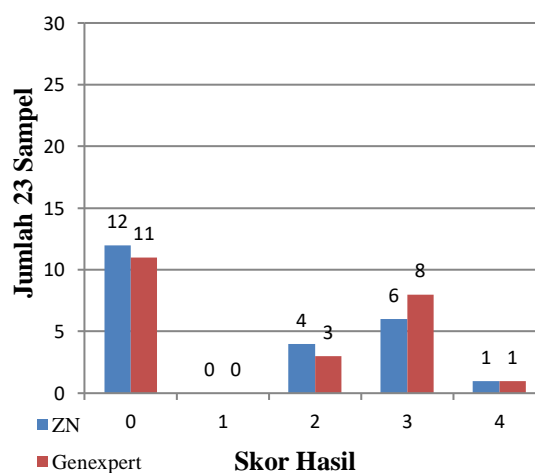
The graph above explains the description of the assessment results from the Zhiel-Neelsen and GeneXpert examination methods based on the many scores obtained in each method. The zero number (0) shows the examination with negative results on Zhiel-Neelsen and MTB Not Detected on GeneXpert, number one (1) shows the scanty results on Zhiel-Neeslen and MTB Detected Very

Low on GeneXpert, number two (2) shows a positive result 1 on Zhiel-Neelsen and MTB Detected Low on GeneXpert, number three (3) shows a positive result of 2 on Zhiel-Neelsen and MTB Detected Medium on GeneXpert and number four (4) shows a Positive 3 result on Zhiel-Neelsen and MTB Detected High on GeneXpert .
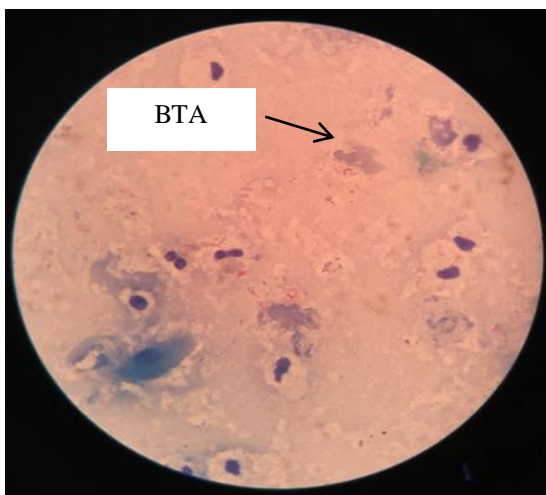
Picture 2.

Negative BTA field results
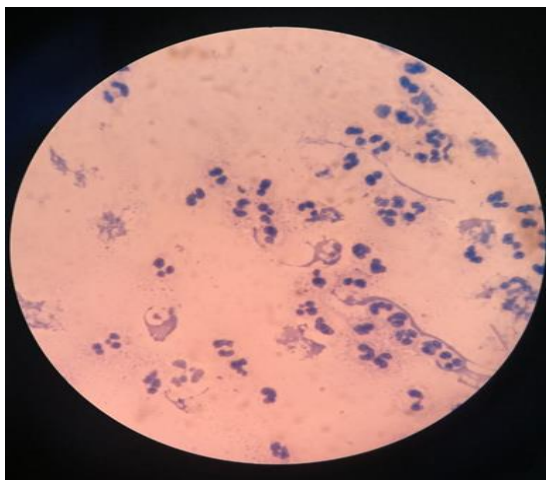
Negative: No Acid-Resistant Bacteria (BTA) were found

Picture 3.

BTA positive field of view results

Positive: Found Acid-Resistant Bacteria (BTA)

Picture 4

GeneXpert test negative results

Negative: MTB Not Detected Results

Picture 5

Positive results of the GeneXpert examination

Positive: MTB Detected Medium results

DISCUSSION

TB disease is still one of the health problems that can be life-threatening, so the right method of examination is needed to detect TB disease early in order to reduce the infection rate and reduce the death rate from TB disease.

In this study, the examination was carried out using two methods, namely the staining method to see the presence of AFB by heating using the Zhiel-Neelsen reagent and examination with GeneXpert, the detection method of Mycobacterium tuberculosis, molecular rapid test with the PCR process.

The examination picture of the sputum sample of the suspect patient obtained the results of the examination using the Zhiel-Neelsen staining method for 12 negative samples and 11 positive samples consisting of 3 positive results of 1/23, 2 positive 6/23 and 1 positive as many as 4/23. Then in the examination using GeneXpert, 11 samples were included in the MTB Not Detected category and 12 samples were detected, consisting of 1/23 MTB Detected High category, MTB Detected Medium category 8/23 and MTB Detected Low category 3/23. and there are no samples with MTB Detected Very Low results.

Several categories of results on the Zhiel-Neelsen staining method were shown based on the number of bacteria read during the examination under the microcope with a defined field of view, namely if in 100 fields of fields no Mycobacterium tuberculosis was found, the results would show negative, BTA was found 1 to 9 in 100 fields From the viewpoint of the results shows positive / scanty, BTA is found 10 to 99 in 100 fields of view the results show positive 1, BTA is found 1-10 in one field of view the results show positive 2 (minimum examination of 50 visual fields) and if the AFB is more than 10 in one field view results show positive 3 with a minimum of 20 field of view examination.

Genexpert provides an overview of the results through fluorescence signal measurements and an automatic algorithm to show quantification of the presence or absence of Mycobacterium tuberculosis DNA. While the category of results for the examination using the GeneXpert method can be determined by the number of bacilli in the specimen based on the Ct value (cycle) shown, namely if the Ct value is less than 16 then the result category to be shown is MTB Detected high, Ct values 16 to 22, indicated by MTB Detected medium, Ct values 22 to 28, the results shown MTB Detected low, Ct values more than 28, results shown MTB Detected very low and Ct 0 values, the results shown are MTB Not Detected or bacteria not found / detected .

Based on the results of research on the description of the results of sputum examination using the Zhiel-Neelsen and GeneXpert methods in TB suspects that were carried out at the Kabila public health center, out of 23 samples, 12 samples were found to be negative by examination with the Zhiel-Neelsen method, after the GeneXpert examination, 11 negative results were obtained. sample and there is 1 negative sample examination of the Zhiel-Neelsen staining method which was detected positive (MTB Detected Low) by the GeneXpert examination. So this shows a change in the examination from the results of the Zhiel-Neelsen method to the GeneXpert method. Changes in the results were also obtained in 2 samples with a positive result 1 Zhiel-Neelsen stain examination which was detected positive 2 (MTB Detected Medium) by GeneXpert examination.

Research put forward by Hartina (2018) and Sayumi (2017) also obtained results that were not much different from this research by Hartina (2018) which showed that there were differences at the time of examination, namely getting negative results on microscopic methods as many as 19/30 and positive 18 / 30 compared to the GeneXpert method got negative results as many as 18/30 and positive as many as 19/30[9] and research by Sayumi (2017) contained samples with categoriesnegative as much as 20/30 on the results of microscopic examination and 15/30 samples of MTB not detected (negative) on the results of the examination by GeneXpert[10]. Research conducted by Kurniawan (2016) also obtained the same results where from 40 patients with negative smear pulmonary

TB with the GeneXpert method, 16 were positive (40%) and 24 negative (60%)[11].

Based on the IUATLD (International Union Against Tuberculosis and Lung Disease) scale, the results are negative on microscopic examination if BTA is not found in 100 fields of view. Changes in the examination results from the Zhiel-Neelsen and GeneXpert methods can occur due to differences in the two methods.

Microscopic examination with Zhiel-Neelsen staining can detect M. tuberculosis if the minimum number of germs contained is 5000 germs / ml in the sputum specimen to get a positive result[11]. The amount of mucus tissue will increase the volume of the sample so that it is less likely to be able to take samples that contain M. tuberculosis and the examination can also be influenced by several things, namely the skill of the practitioner himself starting from making sputum smears on the preparations, the quality of the staining and the method of sample processing. The method of collecting and the quality of sputum obtained is not good[12], therefore in order to get a good quality sample it is necessary to educate the patient before the patient collects sputum / sputum samples first.

In the study presented by Van Rie (2013) in a research journal by Kurniawan et al. (2016) which examined suspected cases of TB with negative smear, the sensitivity and specificity of AFB staining were 27% and 99%, while the RT-PCR GeneXpert method was obtained. sensitivity 67% and specificity 99% [13].

GeneXpert is a breakthrough tool for molecular based TB diagnosis using the semi-quantitative Real Time Polymerase Chain reaction Assay (RT-PCR) method which targets the rpoB gene hotspot in M. tuberculosis which is integrated and automatically processes preparations with

deoxyribo nucleic acid (DNA) extraction. in a disposable cartridge[6]. In the GeneXpert examination, an automatic system that integrates the specimen purification process, nucleic acid amplication and target sequence detection is used. The GeneXpert cartridge has Sample Processing Control (SPC) and Probe Check Control (PCC). SPC functions as an adequate process control against target bacteria as well as to monitor the presence of PCR reaction inhibitors, while PCC functions to ensure the rehydration process of reagents, filling PCR tubes in cartridges,

CONCLUSION

Based on the results of examinations with the Zhiel-Neelsen and GeneXpert methods at Kabila Health Center on 23 samples of suspected TB, it can be concluded as follows:

1. The results of examination with the Zhiel-Neelsen method on sputum suspects of TB obtained negative results as many as 12 (52.2%), positive results 1 as many as 4 (17.4%) positive results 2 as many as 6 (26.1%) and positive results 3 as many as 1 (4.3%)

2. Examination results with the GeneXpert method on sputum suspected of TB were 11 (47.8%) of MTB Not Detected, 3 (13.0%) Detected Low, 8 (34.8%) MTB Detected High and 1 (4.3%) MTB Detected High. .

REFERENCES

[1] Puspasari, Scholastica Fina Aryu. 2019. Nursing Care for Patients With Respiratory System Disorders. New Library Press. Yogyakarta.

[2] Irianti, RN T, Kuswandi, Nanang, M. Y, Ratih, AK 2016. Get to know Anti-Tuberculosis. Yogyakarta.

[3] Novel, Sinta S., Wulandari, Asri P., Safitri, Ratu. 2012. Basic

Microbiology Practicum. Trans Info Media. Jakarta

[4] World Health Organization, 2017. Global Tuberculosis Report 2017, Geneva.

[5] Gorontalo Provincial Health Office. 2019. Health Profile of Gorontalo Province. Gorontalo.

[6] Sadek, Sodikin. 2015. Technical Instructions for Tuberculosis Examination Using the GeneXpert Tool. Ministry of Health of the Republic of Indonesia Directorate General of Health Efforts. Jakarta

[7] Muttaqin, Arif. 2008. Textbook of Nursing Care with Respiratory System Disorders. Salemba Medika. Jakarta

[8] Kementerian Kesehatan Republik Indonesia. 2017. Petunjuk Teknis Pemeriksaan TB Menggunakan Tes Cepat Molekuler. Direktorat Jenderal Pencegahan dan Pengendalian Penyakit. Jakarta.

[9] Hartina, A., Dewi S. S, Wilson W. 2018. Analisis Perbedaan Hasil Pemeriksaan Suspek TB Paru dengan GeneXpert dan Mikroskopik. Fakultas Ilmu Keperawatan Dan KesehatanUniversitas Muhammadiyah Semarang.

[10] Sayumi, E., Dewi S. S, Rohmawati E. 2017. Perbedaan Hasil Pemeriksaan Mikroskopik Dan Genexpert Pada Sputum Suspek TB Kambuh. Fakultas Ilmu Keperawatan Dan Kesehatan Universitas Muhamadiyah Semarang

[11] Kurniawan, E., Raveinal, Fauzar, Arsyad, Z. (2016). Nilai Diagnostik Metode "Real Time" PCR GeneXpert Pada TB Paru BTA Negatif. Artike Penelitian Jurnal Kesehatan Andalas. 5(3) : 130-138

[12] Supriyantoro, SpP, MARS. 2012. Standar Prosedur Operasional Pemeriksaan Mikroskopik TB. Direktorat Jendral Bina Upaya Kesehatan, Direktorat Jendral Pengendalian Penyakit Dan Penyehatan Lingkungan. Jakarta.

[13] Van Rie A, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert® MTB/RIF for point-of care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope?. Expert Rev Mol Diagn. 2010;10(7):937-946.