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Agreement test between Ziehl Neelsen staining and GenXpert in adult pulmonary tuberculosis diagnosis

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ABSTRACT

Background: Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (MTB). Most of healthcare facilities in Indonesia still use Ziehl Neelsen (ZN) staining to diagnose pulmonary tuberculosis (TB) rather than GeneXpert MTB/RIF. However, the agreement between both methods is not well established.
Objective: This study aims to observe levels of agreement between ZN staining and GeneXpert MTB/RIF in diagnosing adult pulmonary TB at Dr. Mohammad Hoesin General Hospital (RSMH) Palembang.
Methods: This study was a retrospective comparative analytical study using an agreement test. Its samples were patient specimens for a period of January 2016 - March 2017 which were examined by ZN staining and GeneXpert MTB/RIF to diagnose pulmonary TB at the Clinical Pathology and Microbiology Installation of RSMH Palembang. Its data were obtained from laboratory results of ZN staining and GeneXpert MTB/RIF. Then, Cohen's Kappa was used to measure the levels of agreement between both the assays.
Results: Of 150 specimens, 69.3% were from male patients and 30.7% were from female patients with mean age of 46.71±14.57. Both ZN staining and GeneXpert positivity were 57.3%. 0.05% of positive GeneXpert MTB/RIF were negative ZN smear, while 0.06% of negative GeneXpert were positive ZN smear. The Cohen's Kappa coefficient was 0.893 indicating a very good agreement.
Conclusion: This study found that there was a very good agreement between ZN staining and GeneXpert MTB/RIF in diagnosing adult pulmonary TB at the RSMH Palembang.

Latar Belakang: *Tuberkulosis (TB) merupakan penyakit menular yang disebabkan oleh Mycobacterium tuberculosis (MTB). Sebagian besar fasilitas kesehatan di Indonesia masih menggunakan metode pewarnaan Ziehl Neelsen (ZN) dibandingkan metode yang lebih sensitif, yaitu GeneXpert MTB/RIF dalam mendiagnosis TB paru. Akan tetapi, tingkat kesesuaian antara kedua metode tersebut masih belum banyak diketahui.*
Tujuan: *Tujuan penelitian ini adalah mengetahui tingkat kesesuaian antara pewarnaan ZN dan GeneXpert MTB/RIF dalam mendiagnosis TB paru dewasa di RSUP Dr. Mohammad Hoesin (RSMH) Palembang.*
Metode: *Penelitian ini menggunakan desain uji kesesuaian. Sampel merupakan spesimen pasien periodic Januari 2016 – Maret 2017 yang dilakukan pemeriksaan pewarnaan ZN dan GeneXpert MTB/RIF untuk mendiagnosis TB paru di Instalasi Patologi Klinik dan Mikrobiologi RSMH Palembang. Data berasal dari data laboratorium hasil pewarnaan ZN dan GeneXpert MTB/RIF. Statistik koefisien Cohen's Kappa digunakan untuk mengetahui tingkat kesesuaian antar metode.*

Hasil: *Dari 150 spesimen, 69.3% spesimen berasal dari pasien laki-laki dan 30.7% perempuan dengan rata-rata umur 46.71±14.57 tahun. Positivitas pewarnaan ZN dan GeneXpert MTB/RIF didapatkan sama, yaitu 57.3%. Sebanyak 0.05% spesimen dengan GeneXpert MTB/RIF positif terdeteksi negatif pada pewarnaan ZN, sedangkan 0.06% spesimen dengan GeneXpert MTB/RIF negatif terdeteksi positif pada pewarnaan ZN. Koefisien Cohen's Kappa antar kedua metode didapatkan 0.893 yang berada di tingkat sangat baik.*

Kesimpulan: *Kesimpulan penelitian ini adalah terdapat kesesuaian yang sangat baik antara pewarnaan ZN dan GeneXpert MTB/RIF dalam mendiagnosis TB paru dewasa di RSMH Palembang.*

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by acid-fast bacilli, *Mycobacterium tuberculosis* (MTB). All human organs almost can be infected by the bacilli, but most of them are lungs (pulmonary TB).[1] WHO estimated that there were 10.4 million cases of TB in 2017, and Indonesia was on the third rank with 8% of cases. Then, there were three million TB cases of underdiagnosed cases or detected cases but not reported in the world.[1,2] Indonesia became the 22 countries with the highest TB cases in the world, and in 2013 pulmonary TB prevalence were 0.4% in Indonesia and 0.2% in South Sumatra province.[3,4]

A rapid and accurate diagnostic method is essential for pulmonary TB diagnosis and control in Indonesia. Bacteriological examinations are needed to confirm the diagnosis.[5] Microscopic examination with Ziehl Neelsen (ZN) staining is less sensitive than a rapid molecular test with GeneXpert MTB/RIF. A study of Munir et al. showed that sensitivity of ZN staining to diagnose pulmonary TB with pulmonary specimens was 77.7%, while sensitivity of GeneXpert MTB/RIF was 90.1%.[6] The ZN staining cannot distinguish between drug-sensitive TB and drug-resistant TB and also cannot differentiate between MTB and non-tuberculous mycobacteria (NTM). Otherwise, the GeneXpert MTB/RIF can detect any mutations at the beta subunit of RNA polymerase (rpoB) genes associated with rifampicin resistance with sensitivity of 95% and specificity of 98%. GeneXpert MTB/RIF can differentiate between MTB and NTM as well.[7,8] Despite variations of the diagnostic methods, a gold standard of *Mycobacterium Tuberculosis* infection is culture; however, it takes long time (2-8 weeks) to get the results, and this method is preferably used for suspected drug resistance in patients who fail to respond to treatment or in patients who relapse.[9,10]

Although GeneXpert MTB/RIF assay has a lot of advantages especially in sensitivity and specificity aspects, ZN staining remain a main TB diagnostic method used in resource-limited countries.[11] GeneXpert MTB/RIF tools in Indonesia has not been distributed well as the ZN staining method. Therefore, information of agreement levels between ZN and GeneXpert MTB/RIF staining in diagnosing the pulmonary TB is necessary.

METHODS

Study design and population

This study was a retrospective comparative analytical study using an agreement test. This study was conducted on October-November 2018. Its samples were specimens from patients either with suspected pulmonary TB or with previously diagnosed pulmonary TB on either clinical or bacteriological treatment for a period of January 2016 - March 2017 examined by ZN staining and GeneXpert MTB/RIF to diagnose the pulmonary TB in the Clinical Pathology and Microbiology Installation of RSMH Palembang. The data of this study were secondary data obtained from laboratory results. Minimum sample size was calculated based on sample size of agreement test formula.[12] The samples were chosen with a simple random sampling technique.

Inclusion criteria for this study were patients with age above 18 years old, laboratory results of ZN staining and GeneXpert MTB/RIF of sputum specimens from the same patients, and laboratory results with complete data of the patients such as name, age, gender and results of ZN staining and GeneXpert MTB/RIF. Specimens from TB/HIV coinfection patients, very low results of GeneXpert MTB/RIF, invalid results, error

results, no results, and indeterminate results of rifampicin sensitivity RIF resistance were exclusion criteria of this study. ZN staining results were considered positive if the ZN positivity level at least 1+, while GeneXpert MTB/RIF results were considered positive if the positivity level at least low.

Statistical analysis

The levels of agreement between ZN staining and GeneXpert MTB/RIF were analysed with Cohen's Kappa coefficient (K). The agreement would be interpreted as poor (K≤ 0.4), fair (K = 0.41 – 0.6), good (K = 0.61 – 0.8), and very good (K≥ 0.81).[13]

Ethical clearance

This study was approved by the Health Research Review Committee of RSMH Palembang and Faculty of Medicine, Universitas Sriwijaya.

RESULTS

Characteristics of the study samples

The mean age of the patients in this study was 46.71±14.57 years old with the most age group of 46-55 years (22.7%). The gender was dominated by male patients. The positivity of ZN staining in this study was same as GenesXpert MTB/RIF (Table 1). The positivity level of ZN 3+ had the highest proportion of 80 ZN positive (smear-positive) specimens, which was equal to 36.3% (Figure 1). Medium GeneXpert MTB/RIF positivity level had the highest proportion of 80 GeneXpert MTB/RIF positive specimens (45%, Figure 2). Of the 80 specimens with positive GeneXpert MTB/RIF, 55 (68.8%) specimens were rifampicin sensitive and 25 (31.3%) specimens were with rifampicin resistance based on GeneXpert MTB/RIF rifampicin sensitivity test (Table 2).

Table 1. Characteristics of samples

Characteristics		N (total 150)	%
Gender	Male	104	69.3
	Female	46	30.7
Age (Years)	20 – 25	10	6.3
	26 – 35	30	18.8
	36 – 45	32	20.0
	46 – 55	34	21.3
	56 – 65	27	16.9
	65 – 85	17	10.6
ZN Staining	Positive	80	53.3
	Negative	70	46.7
GeneXpert MTB/RIF			
	Positive	80	53.3
	Negative	70	46.7
ZN Positivity Level	Negative or scanty	70	46.7
	1+	24	16.0
	2+	27	18.0
	3+	29	19.3
GeneXpert MTB/RIF Positivity Level	Negative	70	46.7
	Low	15	10.0
	Medium	36	24.0
	High	29	19.3

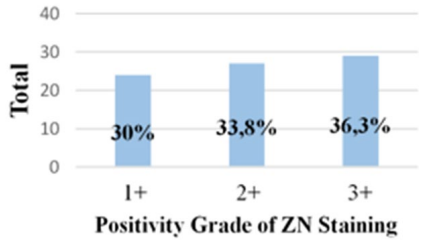

Figure 1. Characteristics of smear-positive Specimens based on ZN positivity level

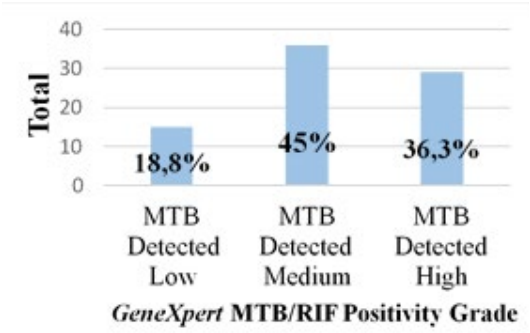

Figure 2. Characteristics of GeneXpert MTB/RIF positive specimens based on GeneXpert MTB/RIF positivity level.

Table 2. Rifampicin sensitivity test of GeneXpert MTB/RIF

Rifampicin Sensitivity	N	%
Sensitive	55	68.75
Resistant	25	31.25
Total	80	100.0

Levels of agreement between ZN Staining and GeneXpert MTB/RIF

A number of 142 specimens had same results in both ZN staining and GeneXpert MTB/RIF, while other 8 specimens had different results. The Cohen's Kappa coefficient was 0.893 indicating a very good agreement (≥ 0.81, Table 3).

Table 3. Levels of Agreement between ZN Staining and GeneXpert MTB/RIF

ZN Staining	GeneXpert MTB/RIF		Total	K
	Positive	Negative		
Positive	76	4	80	0.893
Negative	4	66	70	
Total	80	70	150	

K: Cohen's Kappa coefficient

DISCUSSION

In this study, the mean age of the patients was 46.71±14.57 years old with the most age group of 46-55 years. This finding of this study is in agreement with a WHO statement that TB mostly occurs in adults in a productive age group, even though all ages are at risk of suffering from TB.[14] Then, samples with male gender in this study were more than the female. This finding is in agreement with a meta-

analysis conducted by Horton et al. showing that TB had more prevalence in men than in women.[15] The high prevalence of TB in men and productive age may be due to mobility and work activities as productive workers so that these can increase risks of contracting MTB bacilli from pulmonary TB patients.

Positivity of ZN staining in this study was same as GeneXpert MTB/RIF (53.3%). These results were lower than in a study by Munir et al. reporting that the positivity of ZN staining and GeneXpert MTB/RIF in pulmonary TB suspected patients were 67.5% and 77.4%.[6] This might occur because some specimens in this study came from new patients with suspected pulmonary TB and from patients with suspected multidrug-resistant TB who were undergoing pulmonary TB treatment.

Of 80 smear positive specimens, ZN positivity level with 3+ was 36.3%, level with 2+ was 33.8%, and level with 1+ was 30%. In GeneXpertMTB/RIF, medium positivity level was the highest result found in the positive GeneXpertMTB/RIF specimens (45%), followed by high level (36.3%) and low level (18.8%). Next, 9 of 24 specimens with ZN positivity level of 1+ had medium GeneXpert MTB/RIF positivity level. Higher positivity level of GeneXpert MTB/RIF than ZN positivity level in these 9 specimens might occur because higher sensitivity of GeneXpert MTB/RIF (90.1%) compared to ZN staining (77.7%).[6] Therefore, the number of MTB colonies only detected with 1+ on ZN staining could be detected medium on GeneXpert MTB/RIF.

Of 80 GeneXpert MTB/RIF positive specimens, 55 (68.8%) specimens were rifampicin sensitive and 25 (31.3%) specimens were rifampicin resistant based on GeneXpert MTB/RIF rifampicin sensitivity test. These results weren't in agreement with a study conducted by Sirait et al. at Dr. Hasan Sadikin General Hospital, Bandung reporting that 39.4% of GeneXpert MTB/RIF positive specimens were rifampicin sensitive and 60.6% were rifampicin resistant.[16] This might happen because samples of the study came from suspected pulmonary MDR TB patients, while this study didn't consider specimens taken from suspected TB drug-resistant or drug-sensitive TB patients.

There were 4 specimens with positive smear but negative GeneXpert MTB/RIF. One of ZN staining limitations is its inability to differentiate between MTB and NTM, whereas GeneXpert MTB/RIF can distinguish both of them.[8] Hence, smear positive and negative GeneXpert MTB/RIF need further evaluation regarding the existence of NTM. The other 4 specimens were negative smear but positive GeneXpert MTB/RIF; two of them were low, and the others were high GeneXpert MTB/RIF positivity level. Specimens with low GeneXpert MTB/RIF positivity level and negative smear might have false negative results in ZN staining because the sensitivity of GeneXpert MTB/RIF (90.1%) was higher than ZN staining (77.7%).[6] Factors that can cause false negative results in ZN staining are the absence of internal quality control (IQC) measurement, poor smear thickness and evenness and inappropriate smear size.[17] Moreover, ZN staining microscopy is not sufficient to diagnose pulmonary TB in patients with low mycobacterial load.[18]

Two specimens with high GeneXpert MTB/RIF positivity level but negative smear results might have false positive results in GeneXpert MTB/RIF because these specimens came from patients who were undergoing and negligent in TB treatment. These specimens were evaluated for drug-resistant TB with GeneXpert MTB/RIF. GeneXpert MTB/RIF and mycobacterial DNA can persist for years after antituberculosis treatment in the absence of culturable MTB.[19,21]

The levels of agreement between ZN staining and GeneXpert MTB/RIF in the adult pulmonary TB diagnosis was analysed by using Cohen's Kappa. In this study, the Cohen's Kappa coefficient was 0.893. This value was in a range ≥ 0.81.13 This result was slightly different with a study conducted by Jumbo, Ikuabe, and Ambakederemo at TB Referral Hospital, Yenagoa, Nigeria, finding that the level agreement between the two methods was only "fair" with Cohen's Kappa coefficient of 0.55.[22]

The results of ZN staining are significantly dependent on IQC, the number of leukocytes in sputum and the quality of sputum smears.[17,23] In the study, the authors mentioned that the quality of sputum used in the study in Nigeria was poor so that 21% of negative smear specimens were detected positive in GeneXpert MTB/RIF. Another factor that might have caused this was that the study didn't exclude specimens from paediatric patients and TB/HIV coinfected patients who often showed paucibacillary features.[11,22] Meanwhile, this current study only used specimens from adult patients with one of exclusion criteria, especially TB/HIV coinfected patients.

The very good agreement between ZN staining and GeneXpert MTB/RIF suggests that ZN staining still remain a reliable diagnostic method in diagnosing the adult pulmonary TB in health care facilities that do not have GeneXpert MTB/RIF while considering preanalytical, analytic and post-analytic aspects. This study has some limitations as specimens obtained during treatment and after TB treatment that might influence the GeneXpert MTB/RIF results were not excluded. Then, all scanty ZN staining specimen were considered as smear negative.

CONCLUSION

It could be concluded that there was a very good agreement between ZN staining and GeneXpert MTB/RIF in the adult pulmonary TB diagnosis at RSMH Palembang with a Cohen's Kappa coefficient of 0.893. This suggests that ZN staining still remain a good diagnostic method in diagnosing the adult pulmonary TB in health care facilities that have no GeneXpert MTB/RIF.

CONFLICT OF INTEREST

The authors declared that there wasn't any conflict of interests.

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