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Original Article

Validity of Method for MTBC and NTM Detection in FNAB Specimens from Tuberculous Lymphadenitis Using Microscopy, XPERT MTB / RIF and Culture Method

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ABSTRACT

Mycobacterium tuberculosis and Nontuberculous Mycobacteria usually cause infection in tuberculous lymphadenitis. To improve accuracy of the detection MTB and NTM bacteria it is necessary to select valid methods. This study aims to compare validity of diagnostic methods from FNAB specimens for determining tuberculous lymphadenitis patients. a descriptive observational laboratory study involved 35 samples were obtained from tuberculous lymphadenitis patients in Dr. Soetomo Hospital Surabaya East Java. All specimens examined Ziehl-Neelsen staining microscopy, Xpert MTB/RIF , culture method Middlebrook7H10 solid media and MGIT as Gold standard. Identification of MTB dan NTM with SD Bioline TB Ag MPT64 and niacin paper strip BD . Used diagnostic test 2x2 to analyze sensitivity, specificity, negative predictive value and positive predictive value. Ziehl-Neelsen staining microscopy Sensitivity 83,33 % and Specificity 95,65% of , PPV 90,91%and NPV 91,67%, Diagnostic Accuracy 91,43 % . Xpert MTB/RIF Sensitivity 75% and Specificity 95,65% , PPV 90 % and NPV 88 %, Diagnostic Accuracy 88,57 % with 95% CI (Confidence Interval) . Characteristics female dominated 23/35 (65.7%) while Male numbered 12/35 (34.3%), age range distribution of TB lymphadenitis patients is highest in young adults 17 years to 25 years as many as 15/35 (42.9%) the second highest is the age group of 36 years to 45 years by 8/35 (22.9%), Clinicial presentation are mostly lymph node enlargement in cervical 37% patients other locations supraclavicular ,mamae. Clinical symptoms mostly lymphadenopathy 31,5% and other lymphadenopathy with fever. Microscopy method still have the good validity shoul be conjunction with the molecular rapid tests and culture as gold standard in determining the diagnosis of TB lymphadenitis.

Keywords: *Candida albicans; fluconazole; gastric perforation; histopathological; NSAIDs; peritonitis*

ABSTRAK

Mycobacterium tuberculosis dan Non Tuberculous Mycobacteria merupakan penyebab infeksi pada limfadenitis tuberkulosis. Untuk meningkatkan akurasi isolasi bakteri MTB dan NTM diperlukan pemilihan metode yang valid. Penelitian ini bertujuan membandingkan validitas metode diagnostik deteksi MTB dan NTM serta karakteristik pasien limfadenitis TB dari spesimen FNAB . Penelitian dilakukan di Rumah sakit Dr. Soetomo Surabaya jawa timur bersifat deskriptif observasional diperoleh sebanyak 35 sampel FNAB pasien limfadenitis TB . pemeriksaan yang dilakukan adalah Pemeriksaan mikroskopis menggunakan pewarnaan Ziehl-Neelsen, tes cepat molekuler Xpert MTB/RIF, kultur media padat Middlebrook7H10 . Standar emas pada penelitian ini menggunakan metode kultur media cair MGIT. Identifikasi MTB dan NTM dilakukan dengan SD Bioline TB Ag MPT64 dan niasin paper strip BD. Analisis sensitivitas, spesifisitas,nilai duga negatif dan nilai duga positif menggunakan uji diagnostik tabel 2x2 . . Pemeriksaan mikroskopis pewarnaan Ziehl-Neelsen dengan CI (Confidence Interval) memiliki 95% memiliki nilai sensitivitas 83,33 % dan spesifisitas 95,65%, nilai duga positif 90,91%, nilai duga negatif 91,67%, diagnostik akurasi 91,43 %. Metode diagnostik tes cepat molecular Xpert MTB/RIF sensitivitas 75 % , spesifisitas 95,65 %, nilai duga positif 90 %, nilai duga negatif (88 %, dan diagnostik akurasi 88,57 %. Karakteristik perempuan mendonina-

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si sebanyak 23/35 (65.7%) laki-laki berjumlah12/35 (34.3%), 17 tahun sampai 25 tahun sebanyak 15 orang 15/35 (42.9%) terbanyak kedua adalah kelompok usia 36 tahun sampai 45 tahun sebanyak8/35 (22.9%). Pasien dengan gejala benjolan pada leher sebanyak 37%, lokasi lain supraklavikula, mamme. gejala klinis paling banyak mengalami gejala klinis pembesaran kelenjar getah bening saja sebanyak 31,5%,dan gejala klinis lainnya berupa pembesaran kelenjar getah bening dan febris Pemeriksaan metode mikroskopik masih memiliki validitas yang baik , pemeriksaan yang dilakukan dengan dukungan tes cepat molekular dan kultur sebagai gold standar dapat membantu menegakkan diagnosis limfadenitis TB.

Kata kunci: *Candida albicans; fluconazole; gastric perforasi; histopatological; NSAIDs, peritonitis*

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INTRODUCTION

Tuberculosis is an infectious disease that is still a health problem in the world because of the high burden of mortality and morbidity. World Health Organization (WHO) reported in 2017 number of TB cases are currently 254 per 100,000 or 25.40 per 1 million population.[1] Indonesia has the number of new cases 420,994 cases in 2017,[2] in 2018 Estimated 10.0 million range (9.0 – 11,1 million) people ill with TB Incidence of extra pulmonary TB cases in the world estimated increase 14% from total of 6.4 million TB cases in 2017. Detection of Tuberculous lymphadenitis is quite difficult because the symptoms are often not typical, course of infection depends on patient risk factors and definitive diagnostics can be established based on the discovery of microbes causing infection.[3]

This study aims to compare validity of diagnostic methods specimens examined Ziehl-Neelsen staining microscopy, Xpert MTB/RIF, culture method Middlebrook7H10 solid media and MGIT as Gold standard from FNAB specimens for determining tuberculous lymphadenitis patients.

Microscopic examination with Ziehl-Neelsen staining method is first examination to identify acid fast bacilli (AFB) using a binocular microscope but rarely positive results because of paucibacillary nature of MTB bacteria in tissues.[5] Ziehl-Neelsen staining microscopy Sensitivity range 78,3%- 83,33 % 20. Molecular test Xpert MTB/RIF Assay (Cepheid, USA) is a diagnostic tool recommended by WHO and available in various health care facilities in Indonesia, detect DNA MTB as well as mutations in the rpoB gene that cause resistance to rifampicin.[6] The relevant literature.

Culture method from specimen is still a gold standard in growing bacilli with sensitivity range 70% to 80%. Advantage culture examination to avoid risk of false negative results and obtain MTB isolates for identification. Positive results do not always indicate the presence of living or viable microorganisms. A quick and accurate diagnosis is very important so that there is no over diagnose or under diagnose. Moreover patient can get TB treatment immediately and provide other benefits, such as reducing disability and death rates and preventing TB transmission to others.[7]

MATERIALS AND METHODS

This research is a descriptive observational laboratory testing the validity. FNAB spesimens were taken from April 2019 until June 2019 at the anatomical pathology laboratory.

Material culture method using Microbiology Systems-.BD BACTEC™ MGIT 960 System . Identification of MTB dan NTM with SD Bioline TB Ag MPT64 and niacin paper strip from Becton Dickson company.

Method Ziehl-Neelsen staining microscopy procedure using fuchsine staining 3 minutes, alcohol acid solution staining for 30 seconds and methylene blue staining for 30 seconds, Xpert MTB /Rif using aspirate

samples according manufacture ‘s protocol sample reagent was added in a 2:1 ratio to unprocessed falcon tube, incubation 15 minute at room temperature. And add 2 ml material to cartridge and loaded to Genexpert machine. Culture was put up after decontamination samples on media slopes following the standard protocolMicrobiology Systems-.BD BACTEC™ MGIT 960 System. Used diagnostic test 2x2 to analyze sensitivity, specificity, negative predictive value and positive predictive value.

Ethical Clearance This study received approval from the health research ethics committee of Dr. Soetomo Hospital Surabaya with the statement of ethical clearance of 1232 / KEPK / V / 2019.

RESULTS AND DISCUSSION

Demographic data obtained from medical records April to June 2019 most lymphadenitis patients came from Surabaya with 21/35 (60%) and 14/35 outside Surabaya (40%). No data available incidence of tuberculous lymphadenitis disease at the hospital Dr. Soetomo therefore needs further epidemiological research. Characteristics of gender female dominated as many as 23/35 (65.7%) while Male numbered 12/35 (34.3%) in line with other studies women dominated as many as 120 people (58.8%).[8,9, 10]

Female factors are more dominant because of several factors such as differences in biological, hormonal, social, environmental and different behavior from men. Male and female immune systems can be indicative of underlying causes for various patterns of disease in women. Socially in developing countries, women are more vulnerable because they have low economic status, which makes it late for someone to come to a health care center.[10] The age range distribution of TB lymphadenitis patients is highest in young adults 17 years to 25 years as many as 15/35 (42.9%) the second highest is the age group of 36 years to 45 years by 8/35 (22.9%). Based on the background of the work status there were most 16 /35 employees (46%), the second highest was the non working group.

Because some people have been exposed to people who are suspected of having tuberculosis. Other studies also get the same distribution in the age range of 22 years to 44 years due to the productive age affects the high risk of TB.[11] TB lymphadenitis cases can occur 60 to 80% in people with HIV because TB is an opportunistic infection in people with HIV-AIDS and this disease is found so that in patients with suspected TB lymphadenitis should be screened for HIV co-screening.

Clinical presentation lymph node enlargement mostly in cervical 37% patients other locations supraclavicular mamae. Clinical symptoms mostly lymphadenopathy 31,5% and other lymphadenopathy with fever. Clinical symptoms are one of the conditions for establishing a diagnosis of TB lymphadenitis, but in other studies it was found that clinical syndromes cannot be used as a single basis in determining the diagnosis of TB lymphadenitis because there are many variations in results because it is difficult to determine when the patient first experiences a complaint, subjectivity patients various.[12] Mostly lymph node enlargement sites in collie area, Other studies also have the same data which is located in the cervical area.[13]

Totally 35 specimens were examined 11/35 (31.4%) showed positive smear acid fast bacilli (AFB) and 24/35 (68.6%) negative similar to the results of other studies a total of 120 patients affected by TB lymphadenitis as many as 26 samples (21.7%) found positive smear and 94 samples (78.3%) did not find smear,[14] MTB bacilli were rare found in lymph node tissue because of its Paucibacillary nature, low positivity depends on the average number of aspirate aspirates FNAB 1000 to 10,000 / ml sample, Liquid Culture method MGIT and solid culture method showed positive results 12/35 (34,3 %) and negative 23/35 (65,7%) (Figure 1) (Figure 2), to confirm presence of mycobacteria from positive culture using rapid identification tests SD Bioline TB Ag MPT64 (Figure 3) and niacin paper test showed positive MTBC species 10 /12 (84%) and 2/12 (16%) reported as NTM species.

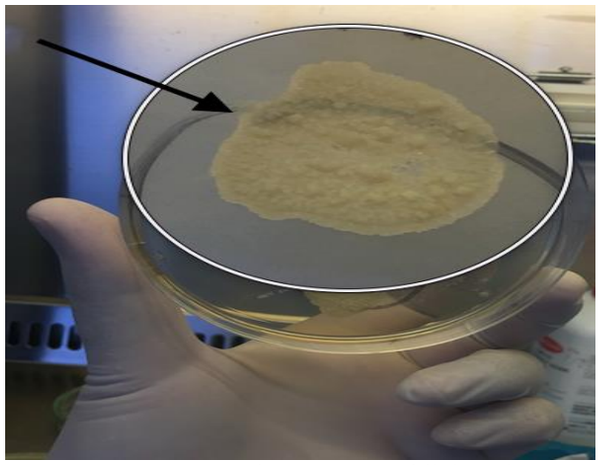

Figure 1. MTB colonies on Middlebrook7H10 medium

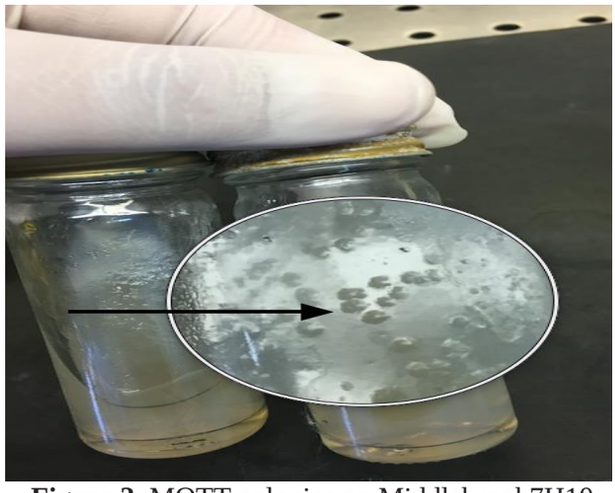

Figure 2. MOTT colonies on Middlebrook7H10 medium

The culture method is a Gold standard examination aimed at isolating MTB bacteria from samples of patients suspected of having TB lymphadenitis. The sensitivity value is quite high because the number of bacteria from 10 to 100 bacilli / ml from concentrated specimens can be detected.[15] Examination of culture methods at the Clinical Microbiology Laboratory Dr. Soetomo uses the Microbiology Systems -.BD BACTEC ™ MGIT 960 System diagnostic tool. Process of decontamination can potentially cause the death of MTB bacilli, too acidic and too alkali conditions can also cause MTB bacilli death and failure to thrive.[16] Other studies comparing the Loweinsten Jensen and Middlebrook 7H10 solid growth media found differences in the growth period of Middlebrook faster than the growth in Loweinsten Jensen media.[17] The sensitivity of the MPT 64 TBAg test kit in differentiating MTB and MOTT 100%.[18]

The results of the examination Molecular test Xpert MTB / RIF Assay (Cepheid, USA) 35 patients with suspected TB lymphadenitis showed that 10/35 (28,69%) patients were positively detected by the M. tuberculosis bacterial gene and negative results in 25/35 (71,4%) . Results of the reading of the detected MTB GeneXpert can be known quantitatively the level of MTB detection using Xpert MTB/RIF tool and categorized as follows: MTB detected low rif resistance not detected 8 samples (80%) and 2 sampels (20%) MTB detected very low rif resistance not detected.

The validity test results in this study used the analysis of the sensitivity and specificity of the FNAB specimens by using the Wilson diagnostic analysis table with 95% confidence interval microscopic method of Ziehl-Neelsen staining (ZN), comparison with the gold standard Culture method MGIT obtained a sensitivity value of 83.33% the ability of Ziehl-Neelsen microscopic staining methods to identify with smear positive results in TB lymphadenitis patients is quite high. Specificity Value of 95.65% with 95% CI means that the ability to find out negative and true results of no AFB in Tuberculous lymphadenitis patients is 95.65%. A positive predictive value of 90.91% means that the probability of AFB being present on microscopic examination if the results of a positive diagnostic test is 90.91%. A negative estimate value of 91.67% means that the probability of not having AFB if the diagnostic test is negative is 91.67%. Diagnostic accuracy of 91.43%. In line with other studies namely sensitivity of 83% and specificity of 98%.[19]

		Culture MGIT	
		Positive	Negative
microscopic	Positive	10	1
	Negative	2	22

Table 1. The result of diagnostic test

Sensitivity value: 83,33%
Specificity Value: 95,65% with 95% CI
positive predictive value of 90.91%: 90,91%
negative estimate value of 91.67%: 91,67%
Diagnostic accuracy of 91.43%.: 91,43 %

The validity test results in this study use the Wilson Wilson diagnostic analysis Table 1 with 95% CI method of molecular Xpert MTB / RIF rapid test comparison with the MGIT culture method as the gold standard. The results of this study obtained a sensitivity value of 75% which means that the ability of the examination of the rapid molecular Xpert MTB / RIF method in identifying MTB bacterial DNA in TB lymphadenitis patients is 75%.Specificity Value of 95.65% with 95% CI means that the ability to find negative and true results of absence of MTB bacterial DNA in TB lymphadenitis patients is 95.65%, positive predictive value of 90% means that the probability of MTB bacterial DNA if positive diagnostic test results is 90%. An estimated negative value of 88% means that the probability of the absence of MTB bacterial DNA if the diagnostic test is negative is equal to 91.67%. Diagnostic accuracy of 88.57%. Specificity (95.65%) in this study is in line with other studies where the specificity of Xpert MTB / RIF is 91%.[20,21,22] Regarding the negative results of the molecular nuclei acid amplification test need procedure of specimen collection . Although the sensitivity value of this study is lower than the specificity value, a high enough specificity value of 95.65% can help in establishing the diagnosis that the patient did not have TB lymphadenitis.

CONCLUSION

TB lymphadenitis diagnostic examination can penot be done only rely on one method in establishing the diagnosis of TB lymphadenitis The distribution of MTB and NTM is very helpful in providing therapy based on the causative agent for TB lymphadenitis infection.

CONFLICT INTEREST

There is no conflict interest of this paper.

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REFERENCES

1. World Health Organization, et al. Mycobacteriology laboratory manual. *Global Laboratory Initiative Advancing TB Diagnosis. Geneva Switzerland: World Health Organization*, 2014
2. Marlina, I. InfoDATIN Tuberkulosis. *Jakarta Selatan*. 2018 Available from: https://pusdatin. kemkes.go.id
3. Purohit M, & Mustafa T. Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. *Journal of clinical and diagnostic research*: *JCDR*. 2015; 9(4): EE01
4. Gandhare, Avinash, et al. Tuberculosis of the lymph nodes: Many facets, many hues. Astrocyte, 2017; 4(2): 80
5. Tadesse M G, Abebe K, Abdissa D, Aragaw K, Abdella A, Bekele L. Rigouts.GeneXpert MTB/RIF assay for the diagnosis of tuberculous LY lymphadenitis on concentrated fine needle aspirates in high tuberculosis burden settings. *PLOS one*. 2015; 10(9): e0137471
6. Kementerian kesehatan RI. Petunjuk teknis pemeriksaan TB menggunakan tes cepat molekuler. 2017 available from : https://www.who.int/tb/ laboratory/mycobacteriology-laboratory-manual.pdf
7. Mertaniasih, Ni Made, et al. Nontuberculous mycobacterial species and Mycobacterium tuberculosis complex coinfection in patients with pulmonary tuberculosis in Dr. Soetomo Hospital,

Surabaya, Indonesia. *International journal of mycobacteriology*. 2017; 6(1): 9-13
8. Singh, Saurabh Kumar; Tiwari, Kamlesh Kumar. Tuberculous lymphadenopathy: Experience from the referral center of Northern India. *Nigerian medical journal: journal of the Nigeria Medical Association*. 2016; 57(2): 134
9. Kamal, Mohammad Shah, et al. Cervical tuberculous lymphadenitis: clinico-demographic profiles of patients in a secondary level hospital of Bangladesh. *Pakistan journal of medical sciences*. 2016; 32(3): 608
10. Pang, Yu, et al. Epidemiology of extrapulmonary tuberculosis among inpatients, China, 2008–2017. *Emerging infectious diseases*, 2019; 25(3): 457
11. Khandkar, Chinmay, et al. Epidemiology of peripheral lymph node tuberculosis and genotyping of M. tuberculosis strains: A case-control study. *PloS one*. 2015; 10(7): e0132400
12. Agatha, I. Gst Ngr Pt Mandela, et al. Uji Klinis Sindroma Klinis Limfadenitis Tuberkulosis Dengan Fine Needle Aspiration Biopsy (Fnab) Sebagai Baku Emas. *E-Jurnal Medika Udayana*
13. Yashveer, J. K.; KIRTI, Y. K. Presentations and challenges in tuberculosis of head and neck region. *Indian Journal of Otolaryngology and Head & Neck Surgery*. 2016; 68(3): 270-274
14. Mitra, Shaila K.; Misra, Rajiv K.; Rai, Priyanka. Cytomorphological Patterns Of Tubercular Lymphadenitis And Its Comparison With Ziehl-Neelsen Staining And Culture In Eastern Up.(Gorakhpur Region): Cytological Study Of 400 Cases. *Journal Of Cytology*, 2017; 34(3): 139
15. Kant, Kamla, et al. Microbiological evaluation of clinically suspected cases of tubercular lymphadenopathy by cytology, culture, and smear microscopy–A hospital-based study from Northern India. *Journal of family medicine and primary care*. 2019; 8(3): 828
16. Chatterjee, Mitali, et al. Effects of different methods of decontamination for successful cultivation of Mycobacterium tuberculosis. *The Indian journal of medical research*. 2013; 138(4): 541
17. Naveen, G.; Peerapur, Basavaraj v. Comparison of the Lowenstein-Jensen medium, the Middlebrook 7H10 medium and MB/BacT for the isolation of Mycobacterium tuberculosis (MTB) from clinical specimens. *Journal of clinical and diagnostic research: JCDR*. 2012; 6(10): 1704
18. Arora, Jyoti, et al. Utility of MPT64 Antigen Detection for Rapid Confirmation Of Mycobacterium Tuberculosis Complex. *Journal of Global Infectious Diseases*. 2015; 7(2): 66
19. Maynard-Smith, Laura, et al. Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC infectious diseases*. 2014; 14(1): 1-15
20. Akhter, Hasina, et al. Diagnosis Of Tuberculous Lymphadenitis From Fine Needle Aspirate By PCR. *Bangladesh Journal of Medical Microbiology*. 2014; 8(1): 2-6
21. Bunger, R., et al. Evaluation of BACTEC Micro MGIT with Lowenstein Jensen media for detection of Mycobacteria in clinically suspected patients of extra pulmonary tuberculosis in a tertiary care hospital at Mullana (Ambala). *J Med Microb Diagn*. 2013; 2(123): 2161-0703.1000123
22. Mertaniasih, Ni Made. *Buku Ajar Tuberkulosis Diagnostik Mikrobiologis*. Airlangga University Press, 2019