The Existence Of Atypical Mycobacterium In Lymph Node Aspiration with Polymerase Chain Reaction (PCR)

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Abstract- Lymphadenitis is an infection of the lymph nodes. This can be caused by infection with Mycobacterium tuberculosis or Atypical Mycobacterium. Infection due to Atypical Mycobacterium is increasing in incidence worldwide, but it is difficult to overcome infections caused by Atypical Mycobacterium. The clinical symptoms of this infection are often the same as tuberculosis so often diagnosed with tuberculosis. Objective: To find out whether there is Atypical Mycobacterium in a fine needle aspiration biopsy sedan confirmed by examination of polymerase chain reaction (PCR) in Indonesia, especially in Medan. This research is a descriptive study with PCR examination with cross sectional approach. The population in this study were all patients with swollen lymph nodes. Examination samples were obtained from aspirate biopsy fine needle aspiration patients. The total number of lymphadenitis cases was 66 cases. Mean age of 26.1 ± 15.1. Where by PCR examination, Atypical Mycobacterium overall represented only 7 cases and male sex dominated 4 cases (12.9%), the most sampling locations were in cervical area 7 cases (13.2%). There is Atypical Mycobacterium in lymph gland aspirates. PCR examination can be recommended to confirm the diagnosis of lymphadenitis in cases of strong suspicion of lymphadenitis tuberculosis because it can be that patients who are not cured by using anti tuberculous agent may be caused by Atypical Mycobacterium

Keywords: Atypical Mycobacterium, nontuberculous Mycobacterium, lymphadenitis, tuberculosis, polymerase chain reaction (PCR).

I. INTRODUCTION

Lymphadenopathy is a condition where there is a change in the size, number and consistency of the lymph nodes. This is a major component of the body's defense system against antigen presenting cells (APC) and serves as the main place where antigen-presenting cells interact with lymphoid cells to produce an adaptive immune response to various foreign antigens including microbes, tumor cells, immune complexes and foreign bodies.[1] Causes of lymphadenopathy are basically caused by an immune response to an infectious agent, or inflammation of an infection involving the lymph nodes. In addition it might be caused by infiltration of neoplastic cells carried by lymphatic circulation or blood to lymph nodes. [2]

Lymphadenitis is an infection of the lymph nodes. This can be caused by infections, viruses and bacteria.[3] Lymphadenitis can be caused by infections with Mycobacterium tuberculosis and Atypical Mycobacterium.[4,5]

Worldwide, tuberculosis (TB) is one of the 10 leading causes of death. Millions of people suffer from TB every year. Drug-resistant TB has always been a public health problem. In 2017, as many as 558,000 people (range, 483,000-639,000) were resistant to rifampicin (Rifampicin Resistant / RR), the most effective first-line drug, and of this number, 82% had multidrug resistant TB (MDR TB). Three countries accounted for almost half of the world's MDR / RR-TB cases: India (24%), China (13%) and the Russian Federation (10%).[6] Indonesia itself is conducting several anti tuberculous agent resistance surveys to get anti tuberculous agent resistance data. [7]

Infection due to Atypical Mycobacterium is increasingly known throughout the world. Difficult to overcome Atypical Mycobacterium infection, in this case long-term therapy with a combination of drugs is needed. However, recurring infections or recurrence of infections caused by Mycobacterium are actually difficult to know. [8]So that Atypical Mycobacterium is easily misdiagnosed as Mycobacterium tuberculosis and sometimes patients are often said to have multidrug-resistant (MDR). [9]We also often get in patients diagnosed with lymphadenitis not responding to targeted antibiotics for bacteria, this could possibly be caused by Atypical Mycobacterium infection.[10,11]

Enlarged lymph nodes are the main target for fine needle aspiration biopsy (FNAB).[12] FNAB in lymph nodes can be used as an initial diagnosis in patients with lymphadenopathy, because it can get immediate results with minimal trauma and fewer complications and lower costs.[13]

To overcome the limitations in differentiating Mycobacterium tuberculosis from Atypical Mycobacterium, an analysis using polymerase chain reaction (PCR) method was developed.[14] The PCR technique allows immediate, sensitive and specific direct diagnosis microbe of identification.[15]

II. MATERIAL AND METHODS

Sample selection

This research is a type of descriptive study to determine the presence of Atypical Mycobacterium in fine needle aspiration by PCR examination with cross sectional approach where each

sample in this study was observed once and only at one time. This research was conducted at the Department of Pathology Anatomics, Faculty of Medicine, University of North Sumatra, located at Jalan Universitas No. 1 Medan, a private hospital or private clinic in the city of Medan. The population in this study were all patients with swollen lymph nodes in the city of Medan. Affordable population are all patients who come to the Department of Pathology Anatomics FK. USU, private hospital or private clinic in the city of Medan. Sample collection was collected by consecutive sampling. All subjects with lymphadenitis who came sequentially and met the inclusion criteria were included in the examination sample. Examination samples were obtained from aspirate patients with fine needle aspiration biopsy.

Inclusion criteria were all patients with enlarged lymph nodes diagnosed with lymphadenitis tuberculosis and all patients with enlarged lymph nodes diagnosed as lymphadenitis.

Lymphadenitis

Tuberculosis lymphadenitis is bacterial lymphadenitis caused by infection with mycobacterium tuberculosis.

Chronic lymphadenitis is a chronic inflammation of the lymph glands that often occurs secondary to a chronic inflammation elsewhere.

Lymph node location

Location of lymph nodes is lymph nodes that are usually palpable in healthy people are cervical, sub-mandibular, axilla, and inguinal.

Cytology diagnosis

Cytology diagnosis is a diagnosis obtained from the results of a fine needle aspiration biopsy cytology. Grouped into:

TB lymphadenitis and non-TB lymphadenitis

Cytological diagnosis is based on three features of tuberculosis cytomorphology:

Type I = epithelioid granuloma without necrosis

Type II = epithelioid granuloma with necrosis

Type III = Necrosis without epithelioid granuloma

Polymerase chain reaction (PCR)

PCR is a way to check DNA from bacteria containing Mycobacterial tuberculosis and Atypical Mycobacterium by amplification and detection in one stage. Grouped into:

Mycobacterium tuberculosis when displayed on 165 base pairs (BP) [16]

Mycobacterium bovis when displayed at 162-375 bp[17]

Mycobacterium avium when displayed at 193-257 bp[18]

Mycobacterium chelonae when displayed at 129 bp[19]

Mycobacterium fortuitum when displayed at 105 bp[19]

Mycobacterium gordonae when displayed at 281 bp[19]

Mycobacterium kansasi when displayed at 221 bp[19]

Mycobacterium paratuberculosis when displayed at 162 bp[17]

Mycobacterium phlei when displayed at 162 bp[17]

Mycobacterium smegmatic when displayed at 162 bp[17]

Mycobacterium xenopi when displayed at 88 bp[19]

Mycobacterial reference is in accordance with ATCC (American Type Culture Collection). PCR amplification according to Mycobacterium avium ATCC 19074 forward GCC GCC GAA ACG ATC TAC, reserve AGG TGG CGT CGAGGA AGA, Mycobacterium bovis ATCC 19210 with ACA AGA CAT GCC TCC CGT, Mycobacterium chelonae ATCC 14472 with AAG CGA CGAGGA AGA, Mycobacterium bovis ATCC 19210 with ACA AGA CAT GCA TCC CGT, Mycobacterium chelonae ATCC 14472 with AAG CGA GACACA AAC ACCA ACA Mycob fortuitum ATCC 6841 with GGG TAA GAC CCA GTG TCT CAA CC, Mycobacterium gordonae ATCC 14470 with CAT GTG TCC TGT GGT CCT, Mycobacterium kansasi ATCC 12478 with CAC GCG GGA TGC GTT TAC GGTG, Mycobacterium paratubulc GCTCC GCC CCC CCC GCC C CGT GAC, Mycobacterium phlei ATCC 11758 with TCC CAG CCA TGC AAC CAG, Mycobacterium smegmatis ATCC 19420 with CGA CCA GCA GGG TGT ATT, Mycobacterium xenopi ATCC 19250 with TCC GAC GAA GTC GTA ACA AGG.

Statistic analysis

In this study the data obtained by researchers will then be processed using descriptive statistics using statistical software and presented in tabular form.

III. RESULTS

In this study, the mean age of patients with lymphadenitis was 26,1 years with a standard deviation of 15,1. Patients with lymphadenitis based on gender are dominated by women by 35 cases (53.0%) while men are only 31 cases (47.0%). Lymphadenitis patients based on the location of the most samples in the cervical area were 53 cases (80.3%), in the sub mandibular area were 7 cases (10.5%) while in axilla each was only 3 cases (4.6%) . Based on the results of a cytological diagnosis, 50 cases (75.8%) were diagnosed with TB lymphadenitis and 16 cases (23.2%) were diagnosed with lymphadenitis. The most cytological diagnosis based on type TB was 28 type TB cases (56.0%) and the second most was type II TB 14 cases (28.0%) and type I TB only 8 cases (16.0%). While negative were 16 cases (24.2%). The results obtained based on the results of PCR, Mycobacterium tuberculosis as many as 31 cases (47%), and the Atypical Mycobacterium obtained a total of 7 cases (10.6%) that is Mycobacterium avium together with Mycobacterium tuberculosis in 3 cases (4.5%), Mycobacterium kansasi which together with Mycobacterium tuberculosis in 1 case (1.5%) and there were 2 cases (3%) found Mycobacterium kansasi, Mycobacterium avium, Mycobacterium xenopi together with Mycobacterium tuberculosis. While there was a Mycobacterium kansasi without the presence of other types of Mycobacterium, there were 1 case (1.5%) and 28 cases were negative (42.4%). Can be seen in table 1.

Table 1 Characteristics of Demographics of Lymphadenitis Patients

Variable	Value
Age (years); mean ± SB	26,1 ± 15,1

Gender (n=66); n%	
Male	31 (47)
Female	35 (53)
Locations (n=66); n%	
Cervical	53 (80,3)
Submandibula	7 (10,6)
Axilla	3 (4,5)
Inguinal	3 (4,5)
Cytology diagnosis (n=66); n%	
Lymphadenitis TB	50 (75,8)
Lymphadenitis	16 (24,2)
Type TB (n=66); n%	
Tipe I	8 (12,1)
Tipe II	14 (21,2)
Tipe III	28 (42,4)
Negative	16 (24,2)
PCR (n=66); n%	
M. tuberculosis (+)	31 (47)
Atypical mycobacterium	7 (10,6)
M. tuberculosis (+), M. avium (+)	3 (4,5)
M. tuberculosis (+), M. kansasi (+)	1 (1,5)
M. tuberculosis (+), M. kansasi (+), M. avium (+), M. xenopi (+)	2 (3)
M. kansasi (+)	1 (1,5)
Negative	28 (42,4)

Gender distribution based on PCR results
Gender distribution based on PCR results can be seen in table 2

Table 2 Genders based on PCR results

Gender	PCR n (66)					
	M. tuberculosis n (31)		Atypical Mycobacterium n (7)		Negative n (28)	
	n	%	n	%	n	%
Female	19	54,3	3	8,6	13	37,1
Male	12	38,7	4	12,9	15	48,4

In this study based on PCR results found in Mycobacterium tuberculosis women in 19 cases (54.3%), and the Atypical Mycobacterium in 3 cases (8.6%) while negative PCR results were in 13 cases (37.1%) . Based on PCR results for male sex, Mycobacterium tuberculosis was found in 12 cases (38.7%), Atypical Mycobacterium there were 4 cases (12.9%) and the negative were 15 cases (48.4%).

Distribution of sampling locations based on PCR results
Distribution based on location can be seen in table 3

Table 3 Distribution of sampling locations based on PCR results

Locations	PCR n (66)					
	M. tuberculosis n (31)		Atypical mycobacterium n (7)		Negatif n (28)	
	n	%	n	%	n	%
Cervical	27	50,9	7	13,2	19	35,8
Submandibula	3	42,9	0	0	4	57,1
Axilla	1	33,3	0	0	2	66,7
Inguinal	0	0	0	0	3	100

In this study based on PCR results obtained in the cervical area obtained 27 cases (50.9%) of Mycobacterium tuberculosis, as well as Atypical Mycobacterium found 7 cases (13.2%) and 19 cases (35.8%) were negative. In sub-mandible Mycobacterium tuberculosis was found in 3 cases (42.9%) while for Atypical Mycobacterium no cases were obtained. And negative based on PCR results in 4 cases (57.1%). In the axilla region Mycobacterium tuberculosis was found in 1 case (33.3%) while atypical mycobacterium was not obtained. And negative were found in 2 cases (66.7%). In the inguinal area, there are no Mycobacterium tuberculosis and Atypical Mycobacterium. While in the inguinal region negative results were obtained in 3 cases (100%).

Distribution of cytological diagnoses confirmed by PCR
The diagnosis of cytology which afterwards is confirmed again by PCR can be seen in table 4

Table 4 Diagnosis of cytology confirmed by PCR

Cytology diagnosis	PCR n (66)					
	M. tuberculosis n (31)		Atypical Mycobacterium n (7)		Negatif n (28)	
	n	%	n	%	n	%
Lymphadenitis TB	24	48	5	10	21	42
Lymphadenitis	7	43,8	2	12,5	7	43,8

In this study the diagnosis of cytology with tuberculosis lymphadenitis was then confirmed by PCR obtained by Mycobacterium tuberculosis in 24 cases (48%) and for Atypical Mycobacterium in 5 cases (10%). While negative were 21 cases (42%). Cytological diagnosis with lymphadenitis was confirmed by PCR with Mycobacterium tuberculosis in 7 cases (43.8%) and Atypical Mycobacterium in 2 cases (12.5%) while negative in 7 cases (43.8%).

The distribution of results diagnosed with lymphadenitis tuberculosis based on the description of the type of cytomorphology confirmed by PCR

Cytological diagnoses diagnosed by tuberculosis lymphadenitis by type confirmed by PCR can be seen in table 5

Table 5 Lymphadenitis tuberculosis based on cytomorphological type images confirmed by PCR

Type TB	PCR n (66)

	M. tuberculosis n (31)		Atypical mycobacterium n (7)		Negatif n (28)	
	n	%	n	%	n	%
Type I	2	25	0	0	6	75
Type II	7	50	1	7,1	6	42,9
Type III	15	53,6	4	14,3	9	32,1
Negative	7	43,8	2	12,5	7	43,8

In this study, Mycobacterium tuberculosis was found to be at most type III in 15 cases (53.6%), Atypical Mycobacterium in 4 cases (14.3%) and negative in type III in 9 cases (32.1%). In type II Mycobacterium tuberculosis was found in 7 cases (50%) and Atypical Mycobacterium in 1 case (7.1%) and negative in 6 cases (42.9%). In type I Mycobacterium tuberculosis was found in 2 cases (25%) and Atypical Mycobacterium was not found while a negative one was found in 6 cases (75%). And negative lymphadenitis TB after being confirmed by PCR found 7 cases (43.8%) Mycobacterium tuberculosis 2 cases (12.5%) Atypical Mycobacterium and negative there were 7 cases (43.8%).

IV. DISCUSSION

Lymphadenitis is often caused by Mycobacterium tuberculosis and Atypical Mycobacterium. [4,5]However, isolation is rarely done for Mycobacterium tuberculosis with Atypical Mycobacterium.[5] Overall patients diagnosed cytologically with lymphadenitis were 66 patients, 50 of whom were lymphadenitis tuberculosis and 16 patients were chronic lymphadenitis. Lymphadenitis patients in this study were most common in women 35 cases (53%) compared to men 31 cases (47%) (table 1), this is in accordance with the research of Rezeki et al., In his study taken from the paraffin block specimen there were more women than men, namely 1.1: 1.[5] and in the Desere et al. [20]study, it was also found that there were more than 80 women compared to 54 cases taken from culture. The most frequent sampling locations were found in the cervical area, according to the study of Kaur et al., [21]In India as much as 80% of cases and according to the research of Rezeki et al., In Bandung also stated that the cervical area was more prevalent.[5]

Patients based on cytological diagnosis found that more than 50 cases of TB lymphomaitis were diagnosed (75.8%). This is in accordance with the study of Mohaputra et al., In India based on aspiration of fine needle biopsy and culture[22] and In accordance with the research of DSuryadi et al., it was also stated that carrying lymphadenitis tuberculosis was more 38,14% than that obtained from a cytological diagnosis. [23]Lymphadenitis tuberculosis is a disease caused by mycobacterium tuberculosis which can attack various organs outside the lung, which is an inflammatory process in the lymph nodes due to Mycobacterium's activity.

In this study, the diagnosis of cytology based on the most cytomorphological features is type III. This is not in accordance with the research of Das et al., Which says that type II is more common in fine needle biopsy aspiration.[24] And Kaur et al., Also said that the most are type II.[21]

This study based on PCR results obtained at most mycobacterium tuberculosis in 31 cases (47%). This is the same as the study of Bensi et al., In Brazil with culture from sputum, there were more mycobacterium tuberculosis 91 cases while Atypical Mycobacterium was found in only 26 cases.[25] Likewise with the study of Lima et al., Which says that Mycobacterium tuberculosis are more common than Atypical Mycobacterium. Where the research samples of Lima et al., Were obtained from sputum, bronchoalveolar lavage (BAL), urine, from skin lesions with aspiration biopsy, pleural fluid and from bone biopsy.[26]

In the gender distribution based on PCR results, it was found that female was more prevalent in 19 cases (54.3%), while Atypical Mycobacterium was more prevalent in males 4 cases (12.9%). This is not in line with the study of Lima et al., Which found that more men were found in Mycobacterium Tuberculosis in 17 cases (45.9%).[26] While in the study of Prevots et al., In America where samples were taken from sputum and BAL found that the prevalence of Atypical Mycobacterium was dominant in women. [27]While the study of Satyanarayan et al., In Virginia the sex distribution was the same as 50%.[28] Tanaka et al., In Japan, from research taken from sputum and BAL found that 84% of women dominated. All of these studies contradict the author's research where more men than women. [29]

Distribution of sampling locations based on the results of PCR the author has not gotten published data about the sampling locations on lymph node aspirates to see this Atypical Mycobacterium. Likewise, the type of cytomorphological picture of tuberculosis diagnosis confirmed by PCR to see Atypical Mycobacterium to the best of the author's knowledge has not yet found published data.

V. CONCLUSION

There were atypical Mycobacterium in lymph node aspirates in 7 cases (10.6%) where 3 cases (4.5%) had Mycobacterium tuberculosis and Mycobacterium avium, 1 case (1.5%) had Mycobacterium tuberculosis and Mycobacterium kansasi, 2 cases (3%) were Mycobacterium tuberculosis, Mycobacterium kansasi, Mycobacterium avium and Mycobacterium xenopi. While there is one case (1.5%), there is only Mycobacterium kansasi.

The mean age in this study was 26.1 ± with a standard deviation of 15.1. The sexes that predominate in Atypical Mycobacterium are male in 4 cases (12.9%). Based on the location of sampling the most was found in the cervical area in 7 cases (13.2%).

VI. COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

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VII. ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara,

Medan, Indonesia approved this study.

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