The correlation between cytology of tuberculosis through eosinophilic mass containing brown particles feature and various bacterial strain

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Abstract-It has been proved that diagnostic cytology of tuberculosis through eosinophilic mass containing brown particles feature can be used to establish diagnose of tuberculous lymphadenitis. The eosinophilic mass containing brown particles is not a classical hystopathology feature of tuberculous infection. Many patients with TB-HIV (Tuberculosis-Human Immunodeficiency Virus) do not show classical feature of TB on pathology examination. Some studies suggest that there is another Mycobacterium strain relates to cytology feature as a specific lymphadenitis. The objective of this study is to find the correlation between cytology of tuberculosis through eosinophilic mass containing brown particles feature and various bacterial strain including M. tuberculosis, M. avium, and other bacterial strains. This study uses fine needle aspiration cytology as a quick, easy and low cost diagnostic tool to detect tuberculous infection especially in lymph nodes of the neck through eosinophilic mass containing brown particles feature. Some of the aspiration will be continued to be examined by PCR to determine the mycobacteroium strain. The correlation between eosinophilic amorphous mass containing dark brown particles and Mycobacterium tuberculosis and Atypical Mycobacterium is not statistically significant, eventhough there is a statistically significant correlation between eosinophilic amorphous mass containing dark brown particles and all mycobacterium strains. We conclude that eosinophilic amorphous mass containing dark brown can be found on Mycobacterium tuberculosis and other Atypical Mycobacterium strains.

Index Terms-Tuberculosis, cytology, eosinophilic amorphous mass

I. INTRODUCTION

Lymphaneopathy is disease of the lymph nodes, in which they are abnormal in size and consistency.[1] Basically, lymphadenopathy is caused by immune response against infection or inflammatory response to infection involving lymph node. It also might be caused by neoplastic cells that infiltrate lymph nodes through lymphatic or blood circulation system.[2]

Lymphadenitis is an infection in lymph nodes. It is usually because of viral and bacterial infection.[3] Lymphadenitis can be caused by infecton of Mycobacterium tuberculosis and Atypical Mycobacterium.[4,5]

Tuberculosis (TB) is one of top ten causes of death worldwide. Million people suffer from TB each year. There are three countries that contributing almost half of MDR/RR-TB cases globally: India (24%), China (13%) and Russian Federation (10%).[6] Indonesia itself is currently doing survey on antituberculosis drug resistance.[7]

Lymph node enlargement is the main target for fine needle aspiration cytology (FNAC). FNAC of lymph node can be used as an initial diagnostic examination to the patient with lymphadenopathy since it can give a quick result with minimal trauma, less complication and more cost effective.[9]

Developed analysis by using polymerase chain reaction (PCR) method can be used to resolve the limitation of distinguishing between Mycobacterium tuberculosis and Atypical Mycobacterium.[10] PCR enables direct, sensitive and specific diagnose when identifying bacteria.[11]

II. MATERIAL AND METHODS

Sample selection
This study is descriptive and analytic design to find out the Atypical Mycobacterium existence by fine needle aspiration cytology which confirmed by PCR with cross-sectional approach. All samples was collected using consecutive sampling. All subjects with lymphadenitis came in sequence. Sample was obtained by fine needle aspiration biopsy and confirmed by using PCR. The procedure of fine needle aspiration of lymph node is using 10 cc disposable syringe with 23G needle or diameter 0,65 mm and 3 cm length or 9 cm length. The dysposable syringe is attached to comeco syringe pistol holder. Aspiration of the lesion was performed by doing some maneuvers and then making specimen preparation and staining with May Grunewlad Giemsa (MGG). The next step was preparing PCR with conventional method to determine atypical mycobacterium. Reference of mycobacterial was according to ATCC (American Type Culture Collection), PCR amplification had an accordance with Mycobacterium avium ATCC 19074 forward GCC GCC GAA ACG ATC TAC, reverse AGG TGG

CGT CGAGGA AGA, Mycobacterium bovis ATCC 19210 with ACA AGA CAT GCA TCC CGT, Mycobacterium chelonae ATCC 14472 with AAG CGA GTA ACC ACT ACA GA AAC, Mycobacterium fortuitum ATCC 6841 with GGG TAA GAC CCA GTG TCT CAA CC, Mycobacterium kansasi ATCC 12478 with CAC GCG GGA TGC GTT TAC GGTG, Mycobacterium paratuberculosis ATCC 19698 with GGC GTT GAG GTC GAT CGC CCA 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.

III. RESULT

This study revealed 66 patients with lymphadenitis, 50 of them (50/66) were lymphadenitis tuberculosis and 16 patients (16/66) were non specific lymphadenitis (non mycobacterium infection and abscess).

Table 1. Correlation between eosinophilic amorphous mass and Mycobacterium tuberculosis

Cytology feature	Result of PCR				p-value
	M. Tuberculosis (+)		M. Tuberculosis (-)		
	N	%	N	%	
Eosinophilic amorphous mass / Tipe III (+)	15	22,7	13	19	0,356
Eosinophilic amorphous mass(-)	16	24,2	22	33,3	

*Chi Square Test

Based on Chi Square test, there was no significant correlation betweeneosinophilic amorphous mass containing dark brown particles and Mycobacterium tuberculosis.

Table 2. Correlation between eosinophilic amorphous mass and Atypical Mycobacterium

Cytology feature	Result of PCR				p-value
	Atypical Mycobacterium		Non Atypical Mycobacterium		
	N	%	N	%	
Eosinophilic amorphous mass (+)	4	6,1	24	36,4	0,446
Eosinophilic amorphous mass (-)	3	4,5	35	53	

*Fishers Exact Test

Based on Fishers Exact test, there was no significant correlation betweeneosinophilic amorphous mass containing dark brown particles and Atypical Mycobacterium.

Table 3. Correlation between eosinophilic amorphous mass and all Mycobacterium strains

Cytology feature	Result of PCR				p-value
	All strains (+)		All strains (-)		
	N	%	N	%	
Eosinophilic amorphous mass (+)	28	42,4	0	0	<0,001
Eosinophilic amorphous mass (-)	22	33,3	16	24,2	

*Fisher Exact Test

Based on Fishers Exact test, there was significant correlation betweeneosinophilic amorphous mass containing dark brown particles and all Mycobacterium strains.

IV. DISCUSSION

Lymphadenitis is usually caused by Mycobacterium tuberculosis and Atypical Mycobacterium.[12,13] It is not frequent to isolate this Mycobacterium tuberculosis from Atypical Mycobacterium.[13] The entire 66 patients were cytologically diagnosed with lymphadenitis, 50 of them were lymphadenitis tuberculosis and 16 patients were non specific lymphadenitis.

Most patients are cytologically diagnosed as Mycobacterium tuberculosis (50 cases/75,8%). This result corresponds to the study of Mohaputra in India which is based on fine needle aspiration biopsy and culture. This also corresponds to the study of Suryadi which states that lymphadenitis tuberculosis is found 38,14% more by cytology.[14,15] Lymphadenitis tuberculosis is disease of lymph nodes due to Mycobacterium tuberculosis that can involve various extrapulmonary organs, in which is an inflammatory response in lymph nodes as a manifestation of Mycobacterium activity.

Our study revealed 31 cases of Mycobacterium tuberculosis (47%) by using PCR. This result has accordance with the study of Bensi in Brazil by using sputum culture method, which revealed 91 cases of Mycobacterium tuberculosis while only 26 cases of Atypical Mycobacterium.[16] The study of Lima also states that Mycobacterium tuberculosis is more found than Atypical Mycobacterium. The study of Lima obtained the sample from sputum, broncho alveolar lavage (BAL), urine, aspiration biopsy of skin lesions, pleural fluid, and bone biopsy.[17]

Our recent study demonstrates that eosinophilic amorphous mass containing dark brown particles is not only found in Mycobacterium tuberculosis and Atypical Mycobacterium, but also found in all Mycobacterium strains (significant correlation). This has accordance with the study of Lubis which cytologically revealed the structure with eosinophilic amorphous mass containing dark brown particles (eosinophilic amorphous mass feature) in speciments from aspiration biopsy of untreated lymph node TB. This study suggested that eosinophilic amorphous mass can be used to diagnose lymphadenitis TB and to distuingish TB and non TB abscess. This study used 20% kudoh culture medium (Ogawa medium modification) and had 194 samples, 110 cases with eosinophilic amorphous mass and 84 cases without eosinophilic amorphous mass. This study revealed 97% sensitivity, 81% specificity, positive predictive value was 83%, negative predictive value was 96%, 89% accuracy, and 48% prevalence.[18]

Khan in 2013 concluded that FNAC was sensitive test and very specific to diagnose lymphadenitis tuberculosis with 77% sensitivity and 98% specificity. Prasoon and Chikkannaiah reported that eosinophilic structure derived from FNA had significant correlation between eosinophilic amorphous mass and Acid Fast Bacilli (AFB) existence in the lesions of lymphadenitis tuberculosis (58,33% and 55,5%).[19,20]

Muyanja confirmed preparation from aspiration biopsy by using hystopathology examination and revealed 93,1%

sensitivity, 100% specifity.[21] The study of Delyuzar on 95 positive TB cases (eosinophilic amorphous mass feature) by using PCR revealed 98,95% sensitivity, 96,7% specificity, positive predictive value was 96,91%, and negative predictive value was 98,97%.[22]

V.   CONCLUSION

Eosinophilic amorphous mass containing dark brown particles correlates not only with Mycobacterium tuberculosis.
Eosinophilic amorphous mass containing dark brown particles correlates not only with Atypical Mycobacterium.
Eosinophilic amorphous mass containing dark brown particles correlates with all Mycobacterium strains.

COMPETING INTERESTS

The authors have no relevant financial interest in the products or companies described in this article.

ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

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