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Research Article

Eosinophil Cell and Mass Appearance in Atypical Mycobacterium Infection of Lymphadenitis

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ABSTRACT

Introduction: *Atypical mycobacterium* (ATM) is acid fast bacilli not including *tuberculosis* and may had opportunistic feature in environment either in air or soil. While symptoms of ATM infection may look similar with typical tuberculosis, these specific group of disease were harder to treat and would necessitate longer antibiotics consumption. While PCR may readily available to detect ATM, anatomical pathology method such as biopsy may be a cheaper alternative in low-resource settings to differentiate between atypical and typical *mycobacterium* infection. **Aim:** To analyze correlation between eosinophil cells and eosinophil mass with ATM in lymphadenopathy patient. **Methods:** This study is an analytical observational study with cross-sectional design which aimed to review diagnostic abilities of eosinophil cell and mass to detect ATM. Patient would undergo both PCR as gold standard of diagnosis and cytology biopsy aspiration as comparative diagnostic modalities. Data would be presented from SPSS v. 25. **Results:** We collected 70 subjects that fulfill inclusion and exclusion criteria. Most samples were dominated by female in relatively young age. There are 37 patients with *ATM* in which 75,7% patients cytology result shown expression of eosinophil cells and 71,4% patients shown eosinophilic mass. Chi-square test revealed that statistical significance existed between eosinophilic cells with ATM. However, such statistical significance was not found between eosinophilic mass and ATM. **Conclusion:** Eosinophilic cell can be used as alternative diagnostic modalities in diagnosing ATM. Further studies should further examine pathophysiological correlations and diagnostic power.

Keywords: Atypical mycobacterium, Eosinophil, Cytology.

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INTRODUCTION

Atypical mycobacterium (ATM) is an acid-resistant bacterium that does not belong to tuberculosis and is an opportunistic organism that exists in the environment both in water and soil.[1-3] ATM infection has the same clinical symptoms as *Mycobacterium tuberculosis* (MTB) infection. ATM infection is very difficult to treat, even requiring a combination of antibiotics with a very long time. In fact, due to the difficulty and length of treatment, cases of ATM infection are considered as multi drug resistant tuberculosis (MDR-TB) patients.

Lubis et al reported patients with a cytological picture of lymph node aspiration biopsy with MGG staining, showing a structure in the form of a dark speck (DS) with an amorphous mass of fine granular eosinophil (BGGE). These patients cannot be treated with regular antibiotics for 2 (two) weeks, but are successful with anti-tuberculous drugs (OAT). When treated with OAT, the lesions usually shrink or disappear within 6-9 months. However, in some cases this shrinkage may take longer, 1.5 years or more. If these latter cases are examined by PCR, there is growth of germs other than Mycobacterium tuberculosa, namely Atypical mycobacterium. And if these aspiration biopsy preparations are studied further, BGGE is seen, as well as eosinophils and eosinophil masses.[4]

ATM infections have started to increase since 1982, especially in patients with HIV/AIDS and immunocompromised condition. Based on research conducted by Seon-cheol Park *et al* in Korea, there was a significant increase in ATM infections where this study was conducted from 2003-2016. Polymerase Chain Reaction (PCR) is a highly specific test to detect the presence of ATM that can be performed on aspirate smears obtained from swollen lymph nodes. [5,6]

In a preliminary study using PCR on glandular aspirates, Fadhilaturrahmi found cases of ATM infection along with the presence of Mycobacterium tuberculosa. The aim of this study was to determine whether eosinophil cells and eosinophil mass are associated with the presence of ATM infection.

MATERIALS AND METHODS

The design of this study was cross sectional (assessing samples at one specific time), analytical observational, categorical, unpaired, not giving treatment to variables, of cytological preparations from aspirates diagnosed as lymphadenitis that were examined for PCR and diagnosed as atypical mycobacterium infection.This study was conducted at various health centres and Central Laboratory of the Faculty of Medicine, Universitas Sumatera Utara. The study period began in January 2020 until the minimum number of samples was met.

Sampling was carried out by non-probability sampling, namely consecutive sampling technique. Where all samples that meet the inclusion and exclusion criteria are taken sequentially until the minimum sample size is met. The samples examined were cytological preparations that had been diagnosed as lymphadenitis by aspiration biopsy cytological examination.

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The calculation of the sample size was carried out using the Lemeshow formula, where from the research of Lubis (2010) obtained a cytological picture of dark specks as many as 25 out of 100 samples examined so that the P (proportion) value = 0.25 and a picture of dark oval bodies as many as 18 out of 100 samples so that the P value = 0.18.[7] The level of significance used in this study is 0.05 with a 95% confidence interval. From the table Zα = 1.96. The error rate is 10%. From the calculation, the largest value of the minimum sample size is 32 but in this study 70 lymphadenitis cytological preparations with eosinophil cells and eosinophil mass will be taken.

Sample Collection

Lymph node aspiration biopsy is performed using a 10 cc disposible syringe with a No. 23 G needle. The syringe is placed on a pistol-like handle manufactured by Comeco, Sweden. Aspiration biopsy can be performed with the patient lying or sitting depending on the site to be examined. Usually anaesthesia is not required. Aspiration is performed with a fine-needle attached to a disposable syringe mounted on a special handle or pistol grip. This tool allows puncture and suction with one hand while the swollen mass can be held or fixed between the thumb and index finger of the other hand. No. 22 needle (external diameter 0.6 mm) is used.

After aspiration is complete the needle is released and the syringe automatically fills with air. The needle and syringe are reconnected and a slight pressure is applied to spray or drip the aspirate in the needle lumen onto the slide. The aspirate is dripped one drop at the end of the slide, and this is best done by touching the tip of the needle to the slide. The tissue fluid on the glass slide is spread by drawing with another slide or cover glass, just like making a normal blood smear preparation. This smear preparation can be air dried and then put into 96% alcohol fixative.

In each case 2 (two) preparations were made: Aspirate I was smeared on a slide then air dried and stained with May Grunewald Giemsa (MGG) and the remaining aspirate was fixed and followed by PCR examination. Aspirate I preparations were diagnosed to determine whether or not these aspirates contained eosinophil cells and eosinophil masses using an Olympus binocular light microscope.

Sample Aspiration

Observations of biopsy aspirate preparations may include:

1. Lymphoid tissue that is polymorphous, many netrophils, with or without epithelioid cells, Langhans type datia cells.
2. Eosinophilic finely granular necrotic tissue without polymorphonucleated lekocytes.
3. Blotchy eosinophilic finely granular necrotic mass. Blotches are necrotic tissues that are darker in colour compared to the surrounding necrotic tissues with indistinct borders. The population of polymorphonucleus lecocyte cells is very small.
4. Necrotic mass, netrophils are abundant, without blotches.

Eosinophilic masses with dark brown particles are assessed based on the following criteria:

1. Within the eosinophilic mass are cells with round to oval nuclei, relatively large, reddish in colour, recognisable nucleoli, indistinct cytoplasm.
2. Small brownish-black masses, cytoplasm indistinct. Compared to the reddish-blue lymphocyte nuclei, these masses are slightly smaller, browner and darker in colour. The shape is more irregular slightly elongated compared to the lymphocytes.
3. The irregular fine particles or streaks are dark brown in colour.

PCR Technique

The remaining aspirates were examined for PCR by conventional method to determine atypical mycobacterium. Mycobacterial reference according to 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 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 gordonae ATCC 14470 with CAT GTG TCC TGT GGT CCT, 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.

DNA extraction was done with extraction kit. 1,5 mL of microbial suspensions were created by centrifugation of suspensions for 4500 rpm for 4 minutes. Cell lysis solution were then added inside the suspension and vortexed for 10-15 seconds. In the next step, 300 uL of the precipitating solution was added to the mixture and mixed for 3 to 5 seconds and placed at 20o C for 20 minutes. It was then centrifuged at 13.000 rpm for 10 minutes. The result were then gently emptied by inverting the tube and placing it on paper for 2 to 3 s. One ml of wash buffer was added to the resulting precipitate and vortexed for 3–5 s. It was centrifuged at 12,000 rpm for 5 min, then the wash buffer was completely evacuated and placed at 65 ° C for 5 min to dry. The precipitate was completely dissolved by solvent buffer and then vortexed for the last time. Supernatant of the result contains pure DNA

PCR products were electrophoresed using 1 % agarose gel (Merck, Germany). A mixture of 1 λ DNA loading Dye and 5 λ PCR product was loaded in the gel. Electrophoresis was performed in voltage from 120 to 90 v.

Statistical Analysis

All data in this study will be analysed using statistical software. Data will be analysed descriptively to determine the frequency distribution of each research variable. Then proceed with inferential analysis to analyse the relationship between independent and dependent variables. The test used is the Chi square test. The results are declared meaningful with a p value of <0.05. All research results will be displayed in tabulated form.

RESULTS

From this study the number of lymphadenopathy cases was as many as 70 subjects: 32 men, 38 women. Average age of the research subjects were 26.5 years with the youngest being 3 years old and the oldest being 54 years old. Average. Based on the results of observations using PCR, the presence of ATM was obtained in 37 subjects while the presence of ATM was not found in 33 subjects. In subjects confirmed to be infected with ATM, presence of eosinophil cells were as many as 30 subjects and patients confirmed to be infected with ATM with eosinophilic masses as many as 26 subjects. As for patients confirmed to be infected with ATM with the presence of eosinophils and eosinophil masses, there were 23 subjects. For the type of specific microorganism, *Mycobacterium bovis* is the most prevalent agent for patient with lymphadenopathy, namely 24 subjects. From this study it was found that ATM may be caused by more than one type of species as many as 27 subjects. We presented these in Table 1.

After statistical testing, it can be concluded that there is a significant relationship between the presence of eosinophil cells in patients infected with ATM ($p<0.05$). However, there was no significant relationship

Table 1. Characteristics of Patient.

Variables	Parameters
Gender, n (%)	
Male	32 (45,7%)
Female	38 (54,3%)
Atypical mycobacterium presence	
ATM (+)	37 (52,9%)
ATM (-)	33 (47,1%)
Eosinophilic cells	
Positive	53 (75,7%)
Negative	17 (24,3%)
Eosinophilic mass	
Focal increase	15 (21,4%)
Moderate increase	25 (35,7%)
Real increase	10 (14,3%)
No mass	20 (28,5%)
***Mycobacterium* species**	
Mycobacterium fortuitum	16 (23,2%)
Mycobacterium kansasi	13 (18,9%)
Mycobacterium bovis	24 (34,7%)
Mycobacterium avium	16 (23,3%)
PCR results of ATM	
Mycobacterium fortuitum	7 (18,9%)
Mycobacterium bovis	3 (8,1%)
Two species of ATM	20 (54,1%)
Three species of ATM	7 (18,9%)
Total	**70 (100%)**

Table 2. Correlation between eosinophil and ATM.

	ATM (+)	ATM (-)	p-value
Eosinophil cells			
Positive	34 (64,2%)	19 (35,8%)	0,001*
Negative	3 (17,6%)	14 (28,4%)	
Eosinophil mass			
Positive	26 (52%)	24 (48%)	0,82*
Negative	11 (55%)	9 (45%)	

*) Chi-square test

between the presence of eosinophil mass and the incidence of ATM infection (p>0.05). All these results were revealed in Table 2.

DISCUSSION

Lymphadenopathy can be caused by several factors such as infection caused by *Mycobacterium tuberculosis* or ATM. After isolating the ATM gene in patients with lymphadenopathy diagnosed as infection with a total sample of 70 subjects using PCR, 37 subjects were found to be positively infected with ATM where 30 subjects had positive eosinophil cells. While for eosinophil mass with the presence of ATM found 23 subjects. From this study it was also obtained that the subject of *Mycobacterium bovis* type was found more as many as 24 subjects then followed by *Mycobacterium Avium* as many as 16 subjects. While for *Mycobacterium phlei, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium paratuberculosis, Mycobacterium smegmatis, Mycobacterium xenopi* in this study was not found. In this study it can also be seen that Atypical mycobacterium infection is more than one type and the most cases are *Mycobacterium bovis* with *Mycobacterium avium* with a total sample size of 9 subjects. In 1885 Koch's discovered ATM and was considered a pathogenic germ since the 1950s. The incidence of ATM continues to increase worldwide and has become a problem for human health and a cause of disease in the lung and outside the lung.[6,8-12]

ATM will only cause infection in humans under certain conditions. Infection is determined by the virulence of the organism, the level of exposure, and the immune response of the host. To cause infection, it requires disruption of the host's defence system.[6]

Although ATM species vary by geography, *Mycobacterium avium complex* (MAC) is the most frequent infection. [5,8,10,14] MAC is recorded as the most prevalent Mycobacterium in East Asia. Meanwhile, in Indonesia, there is no epidemiological data that records the prevalence of this Mycobacterium-caused disease. [1,3] Based on the theory described above, no research has been conducted in Indonesia on ATM. And from the data obtained, the most cases are Mycobacterium bovis followed by *Mycobacterium avium.*

In this study, eosinophil cells were found mainly in several species of Atipycal mycobacterium even when viewed in aspiration diagnosis mostly together with Mycobacterium tuberculosis as many as 19 subjects. In theory, the appearance of eosinophil cells can be due to exposure to viruses, bacteria and even tumour masses. In addition, it is also said that the appearance of eosinophil cells in the body is due to inflammatory reactions, allergies, the regeneration process of bone marrow, gastrointestinal tract and intestinal tissue, even eosinophil cells can also be found in the process of restructuring adipose tissue. In this study, there is a significant relationship between the presence of eosinophil cells and the presence of Atipycal mycobacterium (almost 50%), it can be said that eosinophil cells are found due to allergic processes or inflammatory processes. The presence of eosinophil cells in aspiration biopsy smears on lymph nodes has never been published. While the eosinophil mass in this study is not as many cases as eosinophil cells so there are no eosinophil masses.

CONCLUSION

Eosinophilic cell can be used as alternative diagnostic modalities in diagnosing ATM. Further studies should further examine pathophysiological correlations and diagnostic power.

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DECLARATION OF PATIENT CONSENT

The authors certify that they have obtained all appropriate patient consent forms.

AUTHOR'S CONTRIBUTION

NWH gave a substantial contribution to the conception and design of the work. NWH gave a substantial contribution of data. NWH, DM, RKK, BYMS, AB, F, APT, D gave a substantial contribution to the acquisition, analysis, or interpretation of data for the work. NWH, DM, RKK, BYMS, AB, F, APT, D had a part in article preparing for drafting or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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