The Association between Tuberculosis Cytology through Eosinophilic Mass with Dark Brown Particles against Various Bacterial Strains

Delyuzar1, Bintang Yinke Magdalena Sinaga2, and Rina Yunita3

1 Departement of Anatomycal Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
2 Departement of Pulmonology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
3 Departement of Mirobiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Keywords: Tuberculosis, cytology, eosinophilic mass.

Abstract: Diagnosis of tuberculosis cytology through eosinophilic mass with dark brown particles has been shown to establish the diagnosis of tuberculous lymphadenitis. The eosinophilic mass with dark brown particles is not a classic histopathological of tuberculosis infection, there are allegations that this picture is related to a decrease in the patient's immune system. Many HIV TB patients do not provide a classic TB picture on pathology examination. Mycobacterium avium is associated with TB infection in HIV AIDS patients. The ability to accurately detect tuberculosis infection is very important in line with USU’s research strategic plan with the specialty in the field of Tropical Science and Medicine in controlling tuberculosis infection, especially in patients with reduced immune system. Fine needle biopsy was performed twice for cytologic and Polymerase Chain Reaction (PCR) examination. Statistical analysis was done using Pearson’s Chi square test. Out of 194 cases, 50% were suspicious for tuberculous lymphadenitis and 51% were proved to be positive M. tuberculosis. There was also a trend association between eosinophilic mass and M. avium. All suspected cases of tuberculous lymphadenitis showed eosinophilic mass on microscopic examination of FNA cytology. Our findings suggest the potential of eosinophilic mass as a new diagnostic criteria for TB. This can be the basis of further research on eosinophilic mass.

1 INTRODUCTIONS

Tuberculosis (TB) is still an Indonesian major problem. Indonesia is the second country in the world to have the most cases (321,308 total cases from 242 million inhabitants, 2011). As the fourth country in the cause of the death TB also ranks in Indonesia. Therefore, it needs to research more deeply both for diagnostics and therapy (WHO, 2016). An increase in human resources is needed, include ability of the health workers to find cases and overcome them to decrease tuberculosis transmissions (Delyuzar, 2006). A fast way to detect M. tuberculosis infection will help accelerate early diagnosis in patients who are clinically suspected tuberculosis patients and immediately followed by appropriate management (Lalvani, 2001). Lymphadenitis TB (LTB) can be performed cytology through fine needle aspiration biopsy (FNAB). Diagnostic criteria that have been used to diagnose tuberculosis cytologically are found epithelioid type histiocyte cells and cells with multiple nuclei of the Langhans type (Ammari, 2003). Sarwar A. (2004) explained that in addition to the Langhans cell (multinuclear giant cell) also contains caseous necrosis. PurohitManju (2007) using PCR as the gold standard received anti-MPT64 immunohistochemistry techniques in TB in the abdomen and lymph nodes of sensitivity, specificity, positive predictive value and negative predictive values were 92%, 97%, 98%, and 85%. Immunochemistry with anti-MPT64 antisera can be done relatively quickly, sensitively, and specifically to establish a diagnosis of TB (Tubbs 2009). To establish a diagnosis of tuberculosis lymphadenitis can also be done culture, smear with ZiehlNeelsen staining in addition to histopathological features both classical and immunohistochemistry (Robbins, 2003).

This study uses gold standard biomolecular examination of PCR, so that it complements each other to assess the accuracy of fine needle biopsy cytology diagnostics on unusual TB features.
2 OBJECTIVES

The aimed of this study is to analyze the association between eosinophilic mass and M. tuberculosis.

3 METHODS

This cross-sectional study was carried out in RSUP HAM Medan/Department of Anatomical Pathology, Faculty of Medicine USU Medan or who came to Anatomical Pathology Department, while the examination according to WHO recommendations, PCR was conducted at the USU Integrated Laboratory. The research was done after getting permission from Ethical Committee of Medical Faculty USU Medan. Specimen from FNA was done using a 23 gauge needle, 10 ml syringe and pistol (Comeco, Sweden). Aspirate was directly smeared and stained with May-GrunwaldGiemsa stain. Second FNA procedure was performed to obtain aspirate for PCR examination.

PCR was used to confirm the presence of M. tuberculosis and prove the cytological diagnosis of the first FNA procedure. The following mycobacterial and nonmycobacterial reference bacterial strains were obtained from the American Type Culture Collection (ATCC; Rockville, Md.) used in PCR amplification and grown according to the instructions of ATCC: M. tuberculosis and M. avium. M. tuberculosis Primers, A pair of 24-base synthetic oligonucleotides that bracket a 165-base region of a gene codes for a 65-kilodalton antigen was synthesized. The sequences of the oligonucleotide primers were (from the 5’ to the 3’ ends) CTAG GTCGGACGGTGAGCCAGG and CATTGCCGAAAGT GATTCTCCGGAT. Another oligonucleotide of 40 bases in length located between the two primers was synthesized to be used as an internal probe, and its sequence was (from the 5’ to the 3’ ends) M. avium Primers, a 427-base region.

Statistical analysis was carried out using SPSS 22 version (SPSS Inc., Chicago) with a 95% confidence interval. Pearson’s Chi square test with significance \( p < 0.05 \) was applied to assess the association between eosinophilic amorphous mass and tuberculous.

4 RESULTS

Out of the total 194 patients with lumps on the neck, 18.6% of cases was clinically diagnosed as abscess, 31.4% as non-specific lymphadenitis, 50% as suspicious for tuberculous lymphadenitis (Table 1).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Clinical diagnosis</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td></td>
<td>36 (18.6%)</td>
</tr>
<tr>
<td>Non-specific lymphadenitis</td>
<td></td>
<td>61 (31.4%)</td>
</tr>
<tr>
<td>Suspicious for tuberculous</td>
<td></td>
<td>97 (50%)</td>
</tr>
</tbody>
</table>

Table 2: Association of eosinophilic mass with M. tuberculosis PCR results.

<table>
<thead>
<tr>
<th>PCR</th>
<th>Eosinophilic mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.TB (+)</td>
<td>Presence</td>
</tr>
<tr>
<td>M.TB (-)</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3: Association of eosinophilic mass with M. avium PCR results.

<table>
<thead>
<tr>
<th>PCR</th>
<th>Eosinophilic mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. avium(+)</td>
<td>Presence</td>
</tr>
<tr>
<td>M. avium(-)</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>36</td>
</tr>
</tbody>
</table>

There was a significant association between eosinophilic mass with M. Tuberculosis \( p \) value < 0.001 (Table 2). There was also a trend association between eosinophilic mass with M. avium (Table 3).

Tuberculosis infection proved positive through PCR in 51% of cases (Figure 1). All suspected cases of tuberculous lymphadenitis showed eosinophilic mass on microscopic examination of FNA cytology (Figure 2).

Figure 1: 165 base pairs indicates the presence of M. tuberculosis and 427 base pairs indicates the presence of M. avium on PCR.
DISCUSSIONS

Patel study (2016), 50% of the samples were non-specific lymphadenitis, thirty-six percent was TB and ten percent was abscess, whereas in this study 194 cases (50%) of eosinophilic mass were suspected of Tuberculosis. As control, 31.44% were non-specific lymphadenitis and 18.56% abscess. PCR examination, as gold standard, found 95 positive samples of M. tuberculosis and 99 negative M. tuberculosis samples.

Microscopically the typical picture tissue of the mycobacterium tuberculosis lesion is granuloma or caseous necrosis. Granulomas are a collection of macrophages (macrophages). Macrophages also called histiocytes can fuse to form multinucleated giant cells, macrophages in granulomas are often called epitheloid. Epitheloid macrophages are different from macrophages, usually because they have an elongated core similar to a shoe sole, the core is larger and the cytoplasm is more pink, this change occurs because the macrophage is activated by the antigen. Granulomas may be accompanied by other components including lymphocytes, neutrophils, eosinophils, multinucleated giant cells and fibroblasts. Actually granulomas are not only caused by M. tuberculosis but also due to leprosy, histoplasmosis, cryptococcosis, coccidioidomycosis, and blastomycosis. Non-infectious granulomas can be found in sarcoidosis, Crohn's disiase, berylliosis, Wagener's granulomatosis, Churg-Staruss syndrome and others. Cytology features that contain many macrophages are the most common reactive, infectious and sarcoidosis processes, other conditions can occur in carcinoma with post obstructive pneumonia, infarct and should be differentiated also with Langerhan cell histocytosis (Renshaw, 2005).

Granulomas in tuberculosis tend to form necrosis (caseatingtubercule) although there is no form of necrosis, accompanied by multinucleated giant cells with a nucleus on the edge on one side to form horseshoe/Langhans giant cell (Underwood, 2009). Krisnan (2001) reported different cytologic features in HIV patients he called negative images with a negative rod shape and blue black ground, with no classic features found in LTB patients.

Lubis (2008) found structures of eosinophilic mass with dark brown particles cytologically in patients clinically untreated with TB treatment. Lisdine's research, et al (2003) using Kudoch's reaction to obtain a spotted eosinophilic fine granular necrotic mass can be used as a basis for diagnosing extrapulmonary tuberculosis with probability values of 97%, 91% specificity and 94% accuracy. From the results of this study means the patches found in the pussy microscopically have meaning meaning, where if the encounter of these spots means that the cause of the lesion tuberculosis germs, while not encountered these spots are not the cause of tuberculosis.

Eliandy (2010) examined the appearance of antigens using rabbit polyclonal to Mycobacterium tuberculosis antibody (ab905), Abcam. The appearance of Mycobacterium tuberculosis was seen in 14 cases with small oval-shaped bodies within macrophages, 21 cases with dark patches, 1 case with non-specific chronic inflammation, and 7 cases with abscesses. Lubis et al (2010), examined the difference in the number of positive IHC displays in lesions with small oval-shaped bodies in macrophages and nonspecific chronic inflammation, and there was a difference in the proportion of positive IHC displays in lesions with dark patches of mass amorphous granular eosinophilic ears and abscesses (Sarwar A et al. 2004). But there are still pros and cons about the use of Immunocytochemistry in this cytology so it needs to be reinforced with other techniques more accurate, researchers use the PCR technique as a gold standard. Raviglione and O'Brien (2010) mentioned that granuloma features are not usually found in HIV-infected patients, whereas granulomas are characteristic of TB lesions.

CONCLUSIONS

There was an associate between eosinophilic mass and M. tuberculosis. It indicates the possibility of this cytologic features as a new diagnostic criteria for tuberculous infection. There was also a trend association between eosinophilic mass and M. avium. It indicates the possibility of this cytologic features for Mycobacteriumtuberculosis and Mycobacterium nontuberculosis infection.
ACKNOWLEDGEMENTS

This study was supported by the Ministry of Research, Technology, and Higher Education Republic of Indonesia, Universitas Sumatera Utara, Research Institution, Number: 27/UN5.2.3.1/PPM/KP-DRPM/2018.

REFERENCES


Delyuzar, Arbaningsih SR, Ruswardi., 2006. Empowerment human resources against tuberculosis in North Sumatera: a FIDELIS initiativeThe International Jornal of Tuberculosis and Lung Disease, 10 (11).


