The Correlation Between Eosinophil And Eosinophilic Amorphous Structure In Tuberculous Lymphadenitis Patients

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Background: Eosinophils have bactericidal properties through phagocytosis, respiratory burst, and cytotoxic protein mobilization if bacterial infection occurs. Even though neutrophils have been reported as predominant phagocytic cells in tuberculosis patients, eosinophils also play important role in it. To date, there have been some researches studied about eosinophil and lung tuberculosis. But, data about the association between eosinophil and eosinophilic amorphous structure in tuberculous lymphadenitis patients hasn't been studied so far.
Objective: To analyze the correlation between eosinophil and eosinophilic amorphous structure in tuberculous lymphadenitis patients.
Material and Method: This analytic study with a cross-sectional study from 69 tuberculous lymphadenitis patients who had their aspiration biopsy done and confirmed by PCR. Data about age, gender, and location were obtained from the medical record. Patients' aspiration samples were stained with May Grunewald Giemsa. After that, the researchers determined whether there was eosinophilic amorphous structure and eosinophil. Results of analysis data were presented in tables. The statistical test used in this study is the Mann-Whitney U test.
Result: This study showed that there was no enough evidence to state that there is a correlation between eosinophil and eosinophilic amorphous structure or could be stated that there was no correlation between eosinophil and eosinophilic amorphous structure (p value= 0.0729). Therefore, further studies were needed to confirm this research.
Keywords: lymphadenitis, tuberculosis, eosinophil, eosinophilic amorphous structure

I. INTRODUCTION

Tuberculous (TB) lymphadenitis is an extrapulmonary *TB* commonly found as inflammatory processes occur in lymph nodes due to Mycobacterium tuberculosis bacteria.[1,2] Based on World Health Organization (WHO), in 2019 there were approximately 10 million TB cases around the world. Geographically, most TB cases were found in South East Asia (44%), Africa (24%), and West Pacific (18%), etc. Eight countries accounted for two-thirds of the TB cases, such as India (27%), China (9%), Indonesia (8%), Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).[3] According to the Ministry of Health of the Republic of Indonesia, an estimated 420,994 new TB cases was found in 2017.[4]

Although diagnostic testing becomes more sophisticated, fine needle aspiration biopsy (FNAB) to date is still considered as the first-line examination in tuberculous lymphadenitis.[5] In 1992, Das et al were the first proposing three main major criteria of TB cytology which is now widely used in some studies, that is type I (epithelioid granuloma without necrosis); type II (epithelioid granuloma with necrosis), and type III (necrosis without epitheloid).[6] In 2008, Lubis et al discovered another feature of tuberculous lymphadenitis, which was dark specks in granular eosinophilic necrotic material in lymph node aspirate.[7] Delyuzar also proved that this eosinophilic structure containing dark chocolate particles could be accurately used as new criteria in determining the diagnose of TB cytology due to high sensitivity and specificity confirmed by polymerase chain reaction (PCR).[8]

Eosinophilic structures appear as pinkish, homogenous, acellular, and well-circumscribed irregular mass on H&E stain, basophilic in Leishman stain, and bluish in Ziehl Nelsen stain. Granuloma formation is a reflection of the delayed-type hypersensitivity reaction. Concerning eosinophilic structure (ES) and acid-fast bacilli (AFB), there are four stages in granuloma [(ES-, AFB+), (ES+, AFB+), (ES+, AFB-), dan (ES-, AFB-).[9,10]

Eosinophils are found in 1-3% of all leucocytes. Most of the human eosinophils (>90%) reside in tissues having substantialcellular turnover and regenerative capacity, including bone marrow, uterus, lymphoid tissues, gastrointestinal tracts (such as esophagus) in normal condition and in a location of wound repair and solid tumor in pathology cases.[11]

M. tuberculosisin guinea pig via a low dose of aerosol showed rapid accumulation of eosinophils in bronchoalveolar lavageand lung granuloma.[12] Driss et aldiscovered that eosinophils could release defensin as a response to Bacillus Calmette-Guerin (BCG) or Mycobacteria cell wall components, which can directly kill BCG *in vitro.*[13] After intranasal BCGinfection, a rat with IFNγR$^{-/-}$ deficient had increased eosinophil levels in blood and eosinophil accumulation in the lung.[14] Eosinophils also communicate with many innate immune cells and serve to bridge innate and adaptive immunity by regulating chemokine and cytokine production (CCL17, CCL22, IL-6) and through antigen presentation.[12] Eosinophils have bactericidal properties through

phagocytosis, respiratory burst, and cytotoxic protein mobilization if bacterial infection occurs.[15]
As mentioned before, there were some researches about eosinophils and lung TB. But, to date, the data correlating eosinophil and eosinophilic amorphous structure in tuberculous lymphadenitis so far still haven't been studied. Therefore, researchers want to further study this correlation.

II. MATERIAL AND METHOD

Sample Selection
Research Type
This study aimed to analyze whether there was a correlation between eosinophil and eosinophilic amorphous structure in tuberculous lymphadenitis with a cross-sectional approach. This study was conducted in the Anatomical Pathology Department of Medical Faculty of Universitas Sumatera Utara Medan, a private Hospital or Clinic in Medan. The samples were obtained by consecutive sampling. Samples were obtained from FNAB aspirates.
Included in the sample were all tuberculous lymphadenitis diagnosed based on PCR. An independent variable was eosinophilic amorphous structure, whilethe independent variable was the existence of eosinophils.

Locations of lymph nodes were neck, submandibular, axillary, and inguinal *regions*. *The product of the* tuberculosisis a 165 bp long DNA fragment.

Eosinophilic amorphous structure refers to dark specks in granular eosinophilic necrotic material background. Eosinophil usually has a nucleus that is segmented into bi- or more lobes connected with thin filament, abundant cytoplasm filled with many reddish-orange granules.[16]
The existence of eosinophils refers to whether there are eosinophils or not seen in a light microscope by counting them at 400x magnification in 10 areas randomly chosen. The highest number is used in this study and categorized into 4 groups: [17]
0 eosinophils= absence of eosinophils
1–4 eosinophils= poor infiltration
5–19 eosinophils= moderate infiltration
≥20 eosinophils= severe infiltration

Statistical analysis
In this study, data obtained by researchers were processed by using statistical software and presented in tables.

III. RESULTS

A total of 69 tuberculous lymphadenitis patients were obtained during this study.

Sample Characterization
Tuberculous lymphadenitis patients were distributed based on gender, age, and location of the lymph nodes (Table 1).

Table 1 Distribution of tuberculous lymphadenitis patients based on gender, age, and location

Sample Characterization		Total (n)	Percentages (%)
Gender			
–	Male	26	37.7
–	Female	43	62.3
Age (Mean±SD)		27.1 ± 12.9	
Location			
–	Cervical	59	85.5
–	Sub Mandibular	4	5.8
–	Axilla	4	5.8
–	Inguinal	2	2.9

The mean age of patients was 27.1 ± 12.9 years (mean ± SD). Most of the patients were female (43 people, 62.3%). Most of the nodules were located in the cervical lymph node (59 people, 85.5%). The rest were 4 (5.8%) in submandibular, 4 (5.8%) in axillary, and 2 (2.9%) in inguinal nodes.

The relation of eosinophilic amorphous structure and eosinophils in tuberculous lymphadenitis is shown in Table 2.

Tabel 2. Distribution of eosinophilic amorphous structure and eosinophils in tuberculous lymphadenitis.

Cytology features		Total (n)	Percentages (%)
Eosinophilic amorphous structure			
–	Absence	32	46.4
–	Presence	37	53.6
The existence of eosinophils			
–	Absence of eosinophils	23	33.2
–	Presence		
○	Poor infiltration		
○	Moderate infiltration	42	60.9
○	Severe infiltration	4	5.8
		0	0

Thirty-seven out of 69 patients had eosinophilic amorphous structure (53.6%) while 42 out of 69 patients had poor infiltration eosinophil (60,9%).

Furthermore, in this study, we also evaluated whether there was a correlation between eosinophils and eosinophilic amorphous structure in tuberculous lymphadenitis (Table 3)

Tabel 3. Cross tab between eosinophils (two groups) and eosinophilic amorphous structure

Eosinophilic amorphous structure		**Eosinophils**				**Total**
		Absence		**Presence**		
		n	**%**	**n**	**%**	
•	Absence	14	43.8	18	56.2	32
•	Presence	9	24.3	28	75.7	37

First, researchers categorize the existence of eosinophils into two groups, ie. absence and, presence. It was found that 18 (56.2%) patients had eosinophils even though no eosinophilic amorphous

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structure was observed. Meanwhile, there were 28 (75.5%) patients show eosinophils in their eosinophilic amorphous structure. Looking at Table 3, there was a tendency that in patients who have no eosinophilic amorphous structure had no eosinophils while those who have the structure, most likely had eosinophils. Unfortunately, there was no significant correlation between eosinophils and eosinophilic amorphous structure, statistically.

Table 4. The correlation between eosinophils and eosinophilic amorphous structure

Eosinophilic amorphous structure	Eosinophil								*P-value**
	Absence		Poor infiltration		Moderate infiltration		Severe infiltration		
	n	%	n	%	n	%	n	%	
Absence	14	43.8	17	53.1	1	3.1	0	0	0.0729
Presence	9	24.3	25	67.6	3	8.1	0	0	

* Uji Mann-Whitney U

There was no correlation between eosinophils and eosinophilic amorphous structure (p value= 0.0729).

IV.DISCUSSION

Sixty-nine tuberculous lymphadenitis patients were taken as samples. This study found about 53.6% of cases had an eosinophilic amorphous structure. It was in agreement with the study done by Chikkannaiah et al.[10] Meanwhile, Lubis et al found more cases (64%).[18] Pandit et al in Chikkannaiah et al described that this eosinophilic structure is a degenerated, acellular granuloma, associated with caseous necrosis, immunohistochemical/cytochemical shows the presence of mycobacterial antigen and they are confined to necrotic lesions.[10] Granuloma formation is a reflection of the delayed-type hypersensitivity reaction. Up to 3 weeks of infection, mycobacterium bacilli are phagocytosed by macrophages. However, this can't kill the organisms so the bacteria proliferate within the macrophages uncontrolled. After 3 weeks, Th1 immune response to M. tuberculosisis mounted up Th1 cells secrete IFN-γ and other cytokines leading to a conversion of macrophage into epithelioid and giant cells forming granuloma.[10] These activated macrophages accumulate around the center of the lesion and subsequently, the macrophages will effectively neutralize tubercle bacilli without causing further tissue destruction. This necrotic material is assumed as caseous necrosis.[9]

If the response activating macrophages weaken, the growth of mycobacterium can only be discontinued by increased delayed-type hypersensitivity reaction, leading to tissue destruction. Furthermore, the lesion tends to enlarge and surrounding tissues become more damaged. At the center of the lesion, caseous material liquefies. This caseous material contains lots of bacilli.[9]

Relating to ES and AFB, there are four stages in granulomas. Initial stage (in people with good immunity), where ES and AFB negative, granulomas are found. As the granulomas degenerate producing caseous necrosis, mycobacterium is observed in the lesion (ES-, AFB+). After that, further degenerated granulomas cause acellularity leading to the formation of ES (ES+, AFB+). Necrosis reduces pH and creates an anaerobic environment leading to the death of organisms. In this condition, AFB negative is formed, but the eosinophilic structure is still demonstrable (ES+, AFB-). Still further, the more increase anaerobic condition, the more ES and AFB will disappear (ES-, AFB-).[10]

Even though neutrophils have been stated as dominant phagocytes in tuberculosis, the role of eosinophils is also important. It has been stated that in M. tuberculosis infection, neutrophils and eosinophil protein plasma are increased. Although neutrophil activation may contribute to tuberculosis, eosinophils don't seem to, but its enzyme eosinophil peroxide (EPO) is detected in individuals infected by tuberculosis. Eosinophil infiltration in the lung and eosinophilia has been associated with M. tuberculosis infection.[19] Some studies also found eosinophil accumulation inf tuberculosis .[12,20] Haftu et al reported a case report of extrapulmonary tuberculosis with eosinophilia in peripheral location and infection.[20]

Even though to date there are some literature studying eosinophilia in tuberculosis, its distinct contribution in regulating the growth of mycobacterium is still unknown. Some reports support the fact that eosinophil cationic proteins are mycobactericidal promoting lysis.[13] Borelli et al discovered that humanEPOinduces surface alteration followed by the lysis of M. tuberculosis.[20] EPO had been proved to catalyze the killing of M. tuberculosis H37Rvwith similar potency to neutrophil myeloperoxidase (MPO).[19]

After exposure to bacteria, C5a or ligand CCR3, eosinophils rapidly release mitochondria DNA. The eosinophil contains granule proteins ECP and MBP and displays antimicrobial activity. In extracellular spaces, mitochondria DNA and granule proteins bind and kill bacteria *in vitro* and *in vivo*.[21] This is supported by Moideen *et al* showing that lung tuberculosis patients have increased MBP and EDN levels, and anti-tuberculosis therapy can reduce granule protein eosinophils.[22]

Besides releasing granule proteins, eosinophils can also synthesize, store in intracellular granules, and rapidly secrete various cytokines, such as IL-12, IFN-γ, IL-4, IL-5, IL-13, RANTES, IL-8, eotaxin, GM-CSF, IL-3, TGF-α, stem cell factor,TNF-α, IL-6, IL-16, IL-2, dan IL-10.[11]

It has been stated that eosinophilia in pulmonary tuberculosis combined with increased absolute B lymphocytes (CD20+) and IL-5 levels under IFN-γ deficiency in the blood may indicate eosinophils' ability in displacing Th1/Th2 equilibrium toward Th2- associated reactions that often contributes to the progression of the pathological process. The authors concluded that the mechanism of eosinophilic blood reaction formation in tuberculosis is mediated by genetically determined hypersecretion of eosinophil- activating mediators (IL-5 dan eotaxin) by blood cells and overexpression of IL-5RA in eosinophil membrane, which in turn contributes to prolonged stay of eosinophils in blood circulation during tuberculosis infection.[19]

Kirman et al discovered that After intranasal BCGinfection, the rat with IFNγR$^{-/-}$ deficient had increased eosinophil levels in blood and eosinophil accumulation in the lung. It was known that rats with IFNγ$^{-/-}$ or receptor IFNγR$^{-/-}$deficient are very vulnerable to be infected by organisms causing tuberculosis. Because blood

dan tissue eosinophilia induced by helminth infection depended on Th2 IL-5 cytokine, the presence of eosinophils in the cell infiltrates at the site of mycobacterium infection strongly indicated that increased IL-5 levels produced*in vivo* during M. Bovis infection without any IFNγ signal. Rats with IFN-$\gamma R^{-/-}$ that are prone to mycobacterium infection can be partially associated due to increased IL-5 levels and an influx of eosinophils to the site of infection. Eosinophils may exacerbate mycobacterium infection by disturbing activation and function of macrophages by providing an intracellular environment promoting the growth of mycobacterium.[14]

IL-5 is the main cytokine regulating the function of eosinophils. During an allergic response, this cytokine stimulates the differentiation of eosinophils from bone marrow cells and thus blood eosinophilia occurs. Blood eosinophils are recruited to tissues by chemoattractants such as C5a, platelet-activating factor, and CC chemokine. Since in tissues, IL-5 prolongs the survival of eosinophils by inhibiting apoptosis. At the location of inflammation, eosinophils release cytotoxic products, like granular proteins and radical oxygen, leading to the destructing of epithelium.[23]

This study proved that there was no significant correlation between eosinophils and eosinophilic amorphous structure (p value= 0.0729). Granuloma formation of tuberculosis is a reflection of delayed-type hypersensitivity reaction. This eosinophilic structure is a degenerated, acellular granuloma, and associated with caseous necrosis.[10] Eosinophils producing mediators of the cellular and humoral immune response could contribute to most of the imbalance of cytokines in tuberculosis infection and maintain the changes in pulmonary tissue destruction.[24]

Kuzovkova et al stated the participation and the possible role of eosinophils in inflammation in chronic pulmonary tuberculosis. They discovered that many eosinophils in lung cavity structure and specific inflammation foci (including granuloma, pneumonia foci) had been associated with higher activity of tuberculosis processes (p<0,001). Besides, lots of eosinophils were also found in fibro-cavernous tuberculosis lung tissues with high inflammation level (p<0,05) with localization in the wall of the capsule of tuberculoma (granulation layer, on the border with necrosis, fibrous layer, p<0,05). This indicated that there is a possible bactericidal activity and stimulating fibrosis of eosinophils.[24]

Kuzovkova et al expressed that there should be found many eosinophils, especially in the eosinophilic amorphous structure. But unfortunately, the number of eosinophils in this research is not so many. Indeed, to date study of the correlation of eosinophils and eosinophilic amorphous structure in tuberculous lymphadenitis is not being done before.

V. CONCLUSION

We highlight that there is no significant correlation between eosinophils and eosinophilic amorphous structure (p value=0.0729).

VI. COMPETING INTERESTS

The authors have no financial interest that is relevant to the product or company that has been explained in this article.

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VII. ETHICAL APPROVAL

Health Research Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia has approved this study.

VIII. REFERENCES

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