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RESEARCH ARTICLE

Microscopic Features of Central Caseous Necrosis in Tuberculosis Lymphadenitis Patients: The Relationship with Lymphocyte Density

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Abstract: Tuberculosis lymphadenitis (TBLN) is the most common extra pulmonary TB (EPTB) case in the world with a range of 30-40% of all EPTB cases. When the body's immune system cannot kill the pathogen, the body will try to contain the pathogen (granuloma). The outer portion of the granuloma becomes calcified and the cells in the centre undergoes necrosis. The formation of central necrosis is associated with the emergence of cellular hypersensitivity with the features of local accumulation of macrophages and lymphocytes. The current study aimed to determine the relationship between the microscopic features of central caseous necrosis with lymphocyte density in patients with tuberculous lymphadenitis. Thirty histopathological slides of TBLN patients were observed under a microscope to see the features of central caseous necrosis and assess the lymphocyte density with the results assessed based on the cut-off point of the median of all lymphocyte mean values. Based on the microscopic examination, 25 samples of central caseous necrosis were found and the other 5 samples were not found to have caseous necrosis. Twenty-six patients had low lymphocyte density, and 4 patients had high lymphocyte density. The results of the Fisher Exact test obtained p = 0.009. There is a significant relationship between the features of central caseous necrosis and lymphocyte density in patients with TBLN.

Keywords: Central caseous necrosis, lymphocyte density, tuberculous lymphadenitis

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INTRODUCTION

An infectious disease called tuberculosis (TB) is the most common infectious cause of mortality worldwide, ranking first among all infectious causes of death (above HIV/AIDS).[1] According to World Health Organization (WHO) based on the Global Tuberculosis Report 2020, as many as 7.1 million people with TB were reported to have just been diagnosed in 2019 and Indonesia ranked the second highest TB incidence in the world after India with around 562.049 cases.[2]

The main source of transmission of *Mycobacterium tuberculosis* (MTB) is still the majority of TB cases.[2] However, it can also affect other areas of the body. Extrapulmonary TB (EPTB) occurred in 14% of the 6.4 million new TB cases in 2017.[3,4] Most cases of EPTB manifested in the pleura, bone, and lymphatics.[5] Tuberculosis lymphadenitis (TBLN) is the most common EPTB case in the world with a range of 30-40% of all EPTB cases.[6]

The most important histopathological features of TB patients are Langerhans giant cells, caseation, and epithelioid cells.[7] Histopathologically, based on the presence of granulomas, it is divided into two types, namely well-defined granulomas with characteristics of epithelioid cells, central tissue cells, fibrous tissue, mantle lymphocytes, and Langerhans giant cells. A diffuse mixture of lymphocytes, histiocytes, and plasma cells defines the loose-form granuloma. This cell population represents the body's immune response to pathogen attack.[8,9]

When intracellular pathogens such as MTB can resist killing by cytotoxic T lymphocytes (CTL) and hyperactive macrophages, the body attempts to contain the pathogen in cellular structures called granulomas that form around infected macrophages.[10] The inner layer of granulomas contains macrophages and CD4+ T cells, while CD8+ T cells comprise the outer layer. The cells in the middle of the granuloma eventually necrotize, while the outer part of the granuloma becomes calcified and fibrotic. Granulomas' continued occurrence indicates that the illness is progressing toward chronicity.[10] The development of central necrosis is associated with the development of cellular hypersensitivity (delayed hypersensitivity) to MTB. Cellular hypersensitivity is described as a local accumulation of macrophages and lymphocytes.[11] Lymphocytes are the main component of the adaptive immune system, a decrease in the number of peripheral lymphocytes can be caused by apoptosis, necrosis, and redistribution of cells that reflect immune system dysfunction. Its reduction can be used as an independent marker of the development of complications of inflammation, necrosis, and sepsis.[12]

Activity hematopoietic has an impact on increasing the number of leukocytes, especially lymphocyte cells such as the number of CD4 + and CD8 + which indicates the ongoing process of one's body defence. The rate at which immune cells react depends on the type of pathogen, the activity of the pathogen, and the resulting damage.[9] Therefore, this study aimed to determine the relationship between the microscopic features of central necrosis and

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lymphocyte density in patients with TBLN.

METHODS

This study is an observational analytic study with a cross-sectional approach to determine the relationship between microscopic features of central caseous necrosis and lymphocyte density in patients with TBLN. This research has been approved by the ethics committee of the Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, under the ethical clearance number **660/KEPK/FKUMSU/2021.**

This research was conducted from June 2021 to January 2022 which includes literature studies on data processing and research results. This research was conducted at the Regional General Hospital Dr Pirngadi Medan (RSUD Dr Pirngadi Medan) with the population in this study, namely histopathology slides of TBLN patients in 2019-2020 which are limited by using inclusion and exclusion criteria. The population of this study was 92 patients who were selected by convenience sampling method and obtained 30 research samples that met the research criteria.

The data in this study were obtained through primary data which was carried out by examining histopathological slides of patients with TBLN and secondary data obtained through medical records of patients with TBLN in 2019-2020 at RSUD Dr Pirngadi Medan. The primary data in this study were data regarding the presence or absence of central caseous necrosis and data on lymphocyte density in TBLN. Assessment of lymphocyte density was carried out by counting the number of lymphocyte cells in five fields of view carried out by microscopic examination with strong magnification (400x) which aims to count the number of lymphocyte cells around the area of necrosis. The researchers will record the number of lymphocytes that have been examined, then determine the mean value and the median value of the overall mean value will be taken as the cut-off point value. Histopathological slide examination to assess lymphocyte density and central caseous necrosis was performed by the same examiner.

Data analysis was carried out using SPSS in the form of a descriptive test of research variables (frequency and distribution) for knowing data characteristics. Next, test the hypothesis with the Fisher Exact. The results of the analysis are presented in the form of tables and narratives.

RESULTS

The number of patients diagnosed with TBLN at Rumah Sakit Umum Daerah (RSUD) Dr Pirngadi Medan in 2019 there were 65 patients and in 2020 there were 27 patients, and there were 30 research samples that met the inclusion and exclusion criteria. The microscopic features of caseous central necrosis and lymphocyte density can be seen in Figures 1 and 2.

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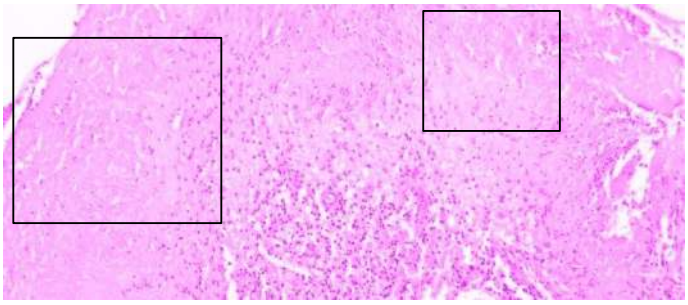

Figure 1. Microscopic features of central caseous necrosis

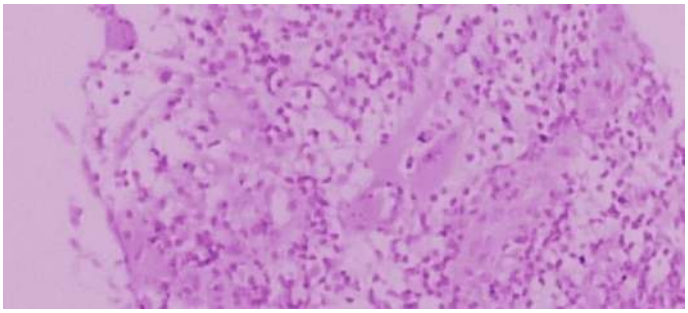

Figure 2. Microscopic feature of lymphocyte density

Table 1. Distribution of patients based on gender and age

	Characteristic	Frequency	Percentage
Gender	Male	43	46.7
	Female	49	53.3
Age	≤ 5	4	4.3
	6 – 15	19	20.7
	16 – 25	23	25.0
	26 – 35	17	18.5
	36 – 45	12	13.0
	46 – 55	6	6.5
	56 – 65	6	6.5
	≥ 65	5	5.4

Table 1 above explains the patients with TB lymphadenitis for the 2019-2020 period at RSUD Dr Pirngadi Medan is more common in females than males with a total sample of 49 (53.3%) and male as many as 43 samples (46.7%). Meanwhile, in terms of age, the most experienced TB lymphadenitis were patients with an age range of 16-25 years, with as many as 23 samples (25%).

Table 2 below shows that the distribution of central caseous necrosis was found in 25 samples (83.3%) and 5 samples (16.7%) did not find central caseous necrosis. The mean value of lymphocytes from 30 samples varied from 19 to 79. As

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the cut-off point value, the median value was 38.0. Lymphocyte density was grouped into low lymphocyte density (lymphocyte mean value 38.0) and high lymphocyte density (lymphocyte mean value >38.0). The distribution of low lymphocyte density was found in 26 samples (86.7%) and as many as 4 samples (13.3%) with high lymphocyte density.

Based on table 3 above obtained the value of Fisher's Exact Test Exact Sig. (2-sided) is 0.009 (P<0.05), which means that there is a significant relationship between the two variables.

Table 2. Distribution of features of central caseous necrosis and lymphocyte density

Microscopic Features		Frequency	Percentage
Central caseous central	Positive	25	83.3
	Negative	5	16.7
Lymphocyte Density	Low	26	86.7
	High	4	13.3

Table 3. The relationship of central caseous necrosis with lymphocyte density

Microscopic Features		Lymphocyte Density		Total	p-value
		Low	High		
Central Caseous Necrosis	**Positive**	24 (80.2%)	1 (3.3%)	25 (83.5%)	
	Negative	2 (6.6%)	3 (9.9%)	5 (16.5%)	0.009
Total		26 (86,8%)	4 (13,2%)	30 (100%)	

DISCUSSION

Mycobacterium tuberculosis infection can affect the body mainly by inhaling organisms containing droplet nuclei in the air. It has been reported that tuberculosis can affect almost every organ in the body.[13] The most frequent extrapulmonary symptom of tuberculosis is lymph node illness (lymphadenitis), which affects more than 25% of all patients.[6,14]

In this study, ninety-two patients with TB lymphadenitis were identified. Forty-nine patients were females (53.3%), and forty-three (46.7%) were males. These results are consistent with the literature which states that females are more likely to get TB than males. As in the results of a study conducted by Garca in Turkey, where females suffer from LNTB more than males with a ratio (of 13:8).[13] There is no concrete explanation for the connection between the female sex and LNTB. However, one study discovered differences in the production of Interleukin-10 and tumour necrosis factor (TNF) across the sexes, indicating that this difference may contribute to susceptibility to tuberculous lymphadenitis. Endocrine, hormonal, socioeconomic, and cultural influences as

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well as CD4+ lymphocyte counts are other explanations for gender disparities.[13,14] Socially, women in poor nations frequently have low socioeconomic and nutritional status, which may have an impact on their ability to respond and their resistance to sickness. Others claimed that while males ignore their disease until it is further advanced, women are more conscious of their appearance and contact health institutions early.[13]

In addition, in this study, most of the LNTB cases occurred in the age range of 16-25 years, with as many as 23 samples (25%). Second rank, age there were 17 samples of 26-35 years old (18.5%), and LNTB patients aged 36-45 years were in the third rank as many as 12 samples (13%). This is to the literature which states that TB lymphadenitis is the most common in young adults between the ages of 15 and 45 years. This is caused by increased activity and lack of personal sanitation at productive age so that the body's immune system is easily decreased and infected with Mycobacterium tuberculosis more quickly.[15,16]

Lymphadenitis TB can be diagnosed based on the patient's clinical manifestations (fever, night sweats, weight loss) and supporting examinations, namely in the form of culture and PCR. The histopathological examination can reveal Langerhans giant cells, epitheloid cells, granulomatous inflammation, calcification, and caseous necrosis which is a form of illustration of the human body's immune response to pathogens.[9,17]

Based on the results of the histopathological examination that has been carried out in this study, it was found that there were features of central caseous necrosis in 25 samples from LNTB patients and 5 other samples did not show any description of necrosis. The formation of central necrosis is associated with the onset of cellular hypersensitivity (delayed hypersensitivity) to Mycobacterium tuberculosis.[11] As in the results of a study conducted by Linawati et al, caseous necrosis was not found in the third week, but by the seventh week, caseous necrosis was seen in the central part of the granuloma. The appearance of necrosis is associated with dysregulation of lipid metabolism due to interactions with Mycobacterium tuberculosis and inflammatory mediators such as TNF- which stimulates the formation of granulomas.[18] The formation of granulomas can occur as a form of a good immune response, whereas in immunocompromised bodies or decreased immunity, granulomas do not form.[18,19] In addition, central caseous necrosis is not always seen on small biopsies, and other granulomatous conditions are possible.[20]

Low and high lymphocyte densities were found to have different proportions in LNTB patients. A total of 26 samples (86.7%) had low lymphocyte density, and 4 samples (13.3%) had high lymphocyte density. The role of lymphocytes, especially T lymphocytes, is as the most important mediator for the body's defence against pathogens and lymphocytes are the main component of the adaptive immune system. In addition, in

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monitoring the course of the disease, the monocyte: lymphocyte ratio can be used. In the healing phase, the number of monocytes decreases, and lymphocytes increase.[21] While the low lymphocyte density can indicate that the immune response is not going well. The risk of extrapulmonary TB increases with decreased or low CD4.[19]

Based on the results of the statistical analysis described in table 3, the results of this study support the initial hypothesis, namely that there is a significant relationship between the appearance of central caseous necrosis and lymphocyte density in patients with LNTB (p-value = 0.009). This explains that the histopathological examination of LNTB patients will find a feature of central caseous necrosis and lymphocyte density with different proportions. High and low lymphocyte density can also be associated with the development of complications or necrosis of the TB lymphadenitis disease. The literature also explains that the decreased lymphocytes can be caused by apoptosis, necrosis, and redistribution of cells that describe the occurrence of immune system dysfunction.[22]

The limitations of this study were carried out without paying attention to the aggravating factors of the disease or comorbidities of the patient which can affect the outcome and severity of LNTB.

CONCLUSIONS

Based on the results of research conducted at RSUD Dr Pirngadi Medan, obtained the number of samples that meet the inclusion and exclusion criteria as many as 30 samples. The results of data analysis carried out with the Fisher Exact test obtained a p-value of <0.005 so it can be concluded that there is a significant relationship between the microscopic picture of central caseous necrosis and lymphocyte density in LNTB patients at RSUD Dr Pirngadi Medan 2019-2020.

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