Diagnostic accuracy of tuberculous lymphadenitis fine needle aspiration biopsy confirmed by PCR as gold standard

DSuryadi[1], Delyuzar[1,*] and Soekimin[1]

[1] Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[*]Corresponding author E-mail: dr_delyuzar@yahoo.com

Abstract. Indonesia is the second country with the TB (tuberculosis) burden in the world. Improvement in controlling TB and reducing the complications can accelerate early diagnosis and correct treatment. PCR test is a gold standard. However, it is quite expensive for routine diagnosis. Therefore, an accurate and cheaper diagnostic method such as fine needle aspiration biopsy is needed. The study aimsto determine the accuracy of fine needle aspiration biopsy cytology in the diagnosis of tuberculous lymphadenitis. A cross-sectional analytic study was conducted to the samples from patients suspected with tuberculous lymphadenitis. The fine needle aspiration biopsy (FNAB)test was performed and confirmed by PCR test.There is a comparison to the sensitivity, specificity, accuracy, positive predictive value and negative predictive value of both methods. Sensitivity (92.50%), specificity (96.49%), accuracy (94.85%), positive predictive value (94.87%) and negative predictive value (94.83%) were in FNAB test compared to gold standard. We concluded that fine needle aspiration biopsy is a recommendation for a cheaper and accurate diagnostic test for tuberculous lymphadenitis diagnosis.

1. Introduction

Tuberculosis (TB) is a major global health problem, which is responsible for the deterioration of the health of millions of people every year. Indonesia is the second largest TB (tuberculosis) burden country in the world. In 2015, it was around 10,4 million TB new cases in the world. 6 countries contributed 60% of new cases which are India, Indonesia, China, Nigeria, Pakistan and South Africa.[1] For overcoming this problem, the capability of a human resource such as health workers and the facilities to find cases and prevent the transmission need to be optimized. Therefore it is necessary to hold a study about the accurate and efficient diagnostic and therapeutic modalities.

Cytology diagnosis of TB is not easy to make. Negative culture result doesn't exclude TB, so it is needed to use other technique as the gold standard, such as histopathology.[2] The grouping of histiocyte cell epithelioid type and multinucleated giant cells from Langhans type are on TB's cytology finding. Besides caseous necrosis of Langhans cell (multinucleated giant cell) is a hallmark in cytology finding.[3,4] On astudy which compares classic histopathology examination and PCR as the gold standard, shows PCR has 92% sensitivity, 37% specificity, 60% positive predictive value and 81% negative predictive value.[5] This study follows American Type Culture Collection's bacteria strain reference which used PCR test for TB and this study used specific primer for M. tuberculosiscomplex.[6]

Majority of extrapulmonary TB is tuberculous lymphadenitis, which usually occurs in the neck

region (scrofula). Tuberculous lymphadenitis is also caused by lymphatic dissemination from extrapulmonary TB primary focus.[7] The percutaneous fine-needle aspiration biopsy (FNAB) usually shows debris, granular necrosis and the combination of inflamed cells, dirty aspiration, such as macrophage mononucleated and multinucleated with coma epitheloid cells (comma shaped). The used of FNAB on lymphadenopathy examination has been a minimally invasive technique which can be accepted and applied widely. It is cheap and accurate as the first line examination of any inflammation condition, granulomatous disease andmalignancy.[8] Nowadays, FNAB is considered as important cytology technique with high diagnostic accuracy, especially when applied to pulmonary cases.[9] Cytology aspiration biopsy is very easy, quick, cheap and suitable for developing the country with limited facility.[10] Different from the previous conclusion, clinician must be consistent in doing M. tuberculosis examination for the lump on neck and FNAB is not recommended for TB cases in Auckland.[11]

2. Methods

This study used analytic cross-sectional based design to determine the accuracy of cytology fine-needle aspiration biopsy method on tuberculous lymphadenitis diagnosis. This study was in Pathology Anatomy Department FK USU, hospital or private clinic in Medan. PCR test was at Integrated Laboratory FK USU. The amount of sample used inthis study was 97 patient with TB characteristics, chronic nonspecific inflammation, and abscess. Study subjects were with nonprobability consecutive sampling. For statistical analyzing the accuracy of FNAB in the detection of tuberculous lymphadenitis, the sensitivity, specificity, positive predictive value and negative predictive value were calculated. All study subjects fulfilled all inclusion and exclusion criteria and undergone FNAB procedure. *M. tuberculosis* was isolated from FNAB specimen for PCR analysis.

Samples were obtained by using needle aspiration biopsy techniques. Aspiration biopsy procedure starting with the skin above the target of the biopsy area. It is swabbed with an antiseptic solution, after locating the mass for biopsy, using palpation, a special needle of very suit diameter is passed into the mass, the needle may be inserted and withdrawn several times, after the needles are placed into the mass, cells are withdrawn by aspiration with a syringe and spread on a glass slide. The cytological evaluation was performed using Giemsa staining.

The following mycobacterial and non-mycobacterial reference bacterial strains were from the American Type Culture Collection (ATCC; Rockville, Md.) for use in PCR amplification and were grown according to the instructions of ATCC:M. tuberculosis ATCC 25177. DNA amplification by PCR. Briefly, 50ng of purified total cellular DNA was amplified with a thermostable Taq DNA polymerase in a thermal cycler (Perkin-Elmer Cetus; Norwalk, Conn.) to establish M. tuberculosis positivity. The 100-,ul DNA amplification reaction mixture contained 10 mM Tris hydrochloride (pH 8.3); 50mM potassium chloride; 1.5mM magnesium chloride; 0.01% gelatin; 20pmol of each of the two primers; 2.5nmol of each of the four deoxyribonucleoside triphosphates; 1U of Taq DNA polymerase (Perkin-Elmer Cetus); and appropriate amounts of specimen DNA, positive control DNA, or negative control DNA. The temperature of the reaction mixture was first raised to 94°C for 20 s, to denature the DNA, and was then cooled down to 63°C for the20s. The temperature of the reaction mixture was then raised to 72°C for 1 min, to extend DNA chain growth. This process was repeated 32 times, with a 10-min incubation at 72°C at the end. One-tenth of the amplified reaction mixture was fractionated electrophoretically in a 2% agarose gel containing 0.5ug of ethidium bromide per ml and was visually examined under UV light for ribboning of DNA of appropriate sizes. If the first result was negative, a portion (usually one-fifth) of the first amplified reaction mixture was amplified for another 32 cycles under the same conditions with freshly supplemented deoxyribonucleoside triphosphates, primers, and Taq DNA polymerase primers. A pair of 24-base synthetic oligonucleotides that bracket a 165-base region of gene codes for a 65-kilodalton antigen was synthesized. The sequences of the oligonucleotide primers were (from the 5' to the 3' ends) CTAG GTCGGGACGGTGAGGCCAGG and CATTGCGAAGT GATTCCTCCGGAT. Another oligonucleotide of 40 bases in length located between the two primers was synthesized to be an

internal probe, and its sequence was (from the 5' to the3' ends) AGCGTAAGTAGCGGGGTTGCCGT CACCCGGTGACCCCCGT.

3. Results

37 cases (38.14%) positive TB cases were found by FNAB and PCR, while 2 cases (2.06%) were negative with PCR test. Three cases were by FNAB and showed a negative result for TB, while PCR showed positive results. There are 55 cases (56.7%) showed the negative result on both tests.

Sensitivity is 92.5%, specificity is 96.49%, accuracy 94.85%, positive predictive value 94.87%, negative predictive value 94.83% and 94.85% accuracy were obtained. Sensitivity shows the ability of an examination to show a positive result, while specificity is the ability of an examination to show negative.

Table 1. Sensitivity and specificity test.

	PCR TB (+)	PCR TB (-)	Total
FNAB TB (+)	37	2	39
FNAB TB (-)	3	55	58
Total	**40**	**57**	**97**

[1]Sensitivity:37 / (37+3) x 100% = 92.50%
[2]Specificity:55 / (55+2) x 100% = 96.49%
[3]Accuracy: (37+55) / (37+2+3+55) x 100% = 94.85%
[4]Positive predictive value:37 / (37+2) x100% = 94.87%
[5]Negative predictive value: 55 / (3+55) x 100% = 94.83%

4. Discussion

In this study, the sensitivity is 92.50%, specificity 96.49%, positive predictive value 94.7%, negative predictive value 94.83% and accuracy 94.85%. A sensitivity value of 92.50% means the probability of a positive FNAB result can detect a TB patient with a positive PCR of 92.50%. A specificity value of 96.49% means that the probability of a negative FNAB outcome can detect a TB patient with a negative PCR of 96.49%. A positive predictive value of 94.87% means that the likelihood of a person suffering from tuberculosis with PCR positive eschar is 94.87% if the patient's FNAB results are positive. A negative predictive value of 94.83% means that the likelihood of a person suffering from TB by PCR negative is 94.83% if the patient's FNAB result is negative. An accuracy of 94.85% means that a positive FNAB result can diagnose 94.85% of patients with both positive and negative PCR results.

This resultshow the same results on Muyanja's research which FNA's sensitivity on TB patient is 93.1% and 100% specificity.[12]Balaji research result showed 98% sensitivity, 100% specificity, 99% accuracy, 100% positive predictive value and 98% negative predictive value on tuberculous lymphadenitis.[13] While Khan concludes that FNA is a very sensitive and specific test to diagnose tuberculous lymphadenitis with 77% sensitivity and 98%specificity.[14]

There are some colleagues' doubts about the results of fine needle aspiration biopsy cytology. This study tries to answer the doubts of other disciplines.

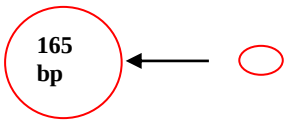

Figure 1. A DNA band of 165 base pairs indicates the attendance of *M. tuberculosis* DNA.

5. Conclusion

After the study was on 97 samples, the results can conclude that the diagnostic accuracy of cytology FNAB, sensitivity, specificity, positive predictive value, thenegative predictive valueof diagnosing tuberculous lymphadenitis are high. The calculation of sensitivity, specificity, accuracy, positive predictive value and negative predictive value shows 92.5% sensitivity, 96.49% specificity, 94.85% accuracy, positive predictive value 94.87% and negative predictive value (94.83%).We concluded that fine needle aspiration biopsy is recommended as a cheaper and accurate diagnostic test for tuberculous lymphadenitis diagnosis.

References

[1] World Health Organization 2016 *Global tuberculosis report 2016* (Geneva: WHO Press) p 13
[2] Gupta S K, Chugh T D, Sheikh Z A and Al Rubah N A 1993 Cytodiagnosis of tuberculous lymphadenitis: A correlative study with microbiologic examination *Acta Cytol.* **37(3)** 329-32
[3] Sarwar A and UlHaque A 2004 Spectrum of morphological changes in Tuberculous lymphadenitis *Int. J. Pathol.* **2** 85-9
[4] Chaudari S, Batra N, Halwal D and Bhat S 2016 FNAC of tubercular lymph node–An alternative to excision biopsy *Indian J. Pathol. Oncol.* **3(2)** 237-41
[5] Singh K K, Muralidhar M, Kumar A, Chattopadhyaya T K, Kapila K, Singh M K, *et al*. 2000 Comparison of in house polymerase chain reaction with conventional techniques for the detection of *M. tuberculosis* DNA in granulomatous lymphadenopathy *J. Clin. Pathol.* **53(5)** 335-61
[6] Pao C C, Yen T S B, You J B, Maa J S, Fiss E H and Chang C H 1990 Detection and identification of *Mycobacterium tuberculosis* by DNA amplification *J. Clin. Microbiol.* **28(5)** 1877-80
[7] Eliandy S, Lubis H M N D and Delyuzar 2011 Hubungan gambaran bercak-bercak gelap (dark specks) pada latar belakang material nekrotik granular eosinofilik dengan kadar CD4 penderita limfadenitis tuberculosis servikalis yang disertai HIV/AIDS *Majalah Patologi Indonesia* **20(03)** 8-14
[8] Patel V K, Sheth R and Shah K 2016 A retrospective study on role of fine needle aspiration cytology in diagnosis of cervical lymphadenopathy *Int. J. Medical Sci. Pub. Health* **5(8)** 1588-91
[9] Diamantis A, Magiorkins E and Koutselini H 2009 Fine-needle aspiration (FNA) biopsy: historical aspects *Folia Histochem. Cytobiol*. **47(2)** 191-7
[10] Ioachim H L and Medeiros L J 2009 Mycobacterial lymphadenitis *Lymph node pathology 4th edition* (Philadelphia: Lippincot Wiilliam& Wilkins) pp 130-6

[11] Harrison A C and Jayasundera T 1999 Mycobacterial cervical adenitis in Auckland: diagnosis by fine needle aspirate *NZ. Med. J.* **112(1080)** 7-9
[12] Muyanja D, Kalyesubula R and Namukwaya E 2015 Diagnostic accuracy of fine needle aspiration cytology in providing a diagnosis of cervical lymphadenopathy among HIV-infected patients *Afr. Health Sci.* **15(1)**
[13] Balaji J, Sundaram S S and Rathinam S N 2009 Fine needle aspiration cytology in childhood TB lymphadenitis *Indian J. Pediatr.* **76(12)** 1241-6
[14] Khan M M, MushTaq S, Mamoon N, Ahmad M and Ahmad S 2013 Morphological spectrum and accuracy of fine needle aspiration cytology in tuberculous lymphadenitis *Gomal J. Med. Sci.* **11** 230-4