https://doi.org/10.21010/Ajidv17i2S.3

GYRB – POLYMERASE CHAIN REACTION AND HISTOPATHOLOGIC CHARACTERISTIC FIGURE POTENTIAL FOR DETERMINING DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS

HERLAMBANG Wahyu [1], MERTANIASIH Ni Made [2,3,6]*, SOEDARSONO Soedarsono[4,6], SANDHIKA Willy [5]

[1]Master Program of Tropical Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Clinical Microbiology, Dr. Soetomo Academic Hospital, Surabaya, Indonesia., [4]Sub-pulmonology of Internal Medicine, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia., [5]Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia., [6]Tuberculosis Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

***Corresponding Author's E-Mail Address:** nmademertaniasih@gmail.com

Article History
Received: Dec. 8th 2022
Revised Received: May 24th 2023
Accepted: June 5th 2023
Published Online: Aug. 1st 2023

Abstract

Background: TB lymphadenitis is still a problem that needs serious treatment. In Indonesia, it was reported that 53% of TB cases were extrapulmonary tuberculosis, with the most cases being Lymphadenitis TB, 11.6%. In children, 43% of extrapulmonary tuberculosis cases are TB lymphadenitis. Diagnosis is quite difficult; a method of determining the diagnosis and appropriate comprehensive treatment is required in managing TB Lymphadenitis.
Materials and Methods: In this study, 15 fine needle aspiration biopsy aspirate samples were subjected to molecular examination using the gyrB–polymerase chain reaction method and histopathological observations using the smear method with hematoxylin-eosin staining. Observation of preparations using a microscope with a magnification of 200x.
Results: The histopathological characteristics of the fine needle aspiration biopsy aspirate showed positive results in 4 out of 15 samples, with epithelioid cells arranged in a characteristic granuloma structure, necrotic debris was visible, and cells joined together to form multinucleated giant cells as an inflammatory response to *Mycobacterium tuberculosis* complex infection. In this study, 6 out of 15 (40%) were detected to be positive in the diagnosis based on molecular detection using a specific target gene gyrB - polymerase chain reaction.
Conclusion: Characteristic features on histopathological examination associated with gyrB - positive polymerase chain reaction on lymphadenitis *fine needle aspiration biopsy* aspirate samples can be used as a determinant diagnosis of tuberculous lymphadenitis.

Keywords: EPTB, FNAB aspirate, *gyrB*, PCR, tuberculosis, tuberculous lymphadenitis

List of Abbreviation: TB : tuberculosis; EPTB: extrapulmonary tuberculosis; FNAB: fine needle aspiration biopsy PCR: polymerase chai reaction; TBL: tuberculous lymphadenitis; NTM: non tuberculous mycobacteria; AFB: acid-fast bacilli; MTBC: Mycobacterium tuberculosis complex; HE: hematoxylin eosin; PMN: polymorphonuclear CT: computed tomography.

Introduction

Tuberculosis is still an infectious disease with high morbidity and mortality globally. Based on reports in 2021, it is estimated that around 10 million people are diagnosed with TB, with a mortality rate of 15% annually (WHO, 2021). Approximately 20% of TB cases are extrapulmonary TB cases (EPTB). EPTB can attack various organs in the body, but generally, 50% of cases are found in the lymph nodes (Baykan *et al*., 2022). In Indonesia, it was reported that 53% of TB cases were EPTB, with the highest number of tuberculous lymphadenitis (TBL) cases being

reported at 11.6%. It was also reported that 45% of pediatric EPTB patients were diagnosed with tuberculous lymphadenitis (Soekotjo *et al.*, 2019; Anggraini and Oktora, 2021).
EPTB can be a primary manifestation if the organ is the initial site of infection, generally caused by non-tuberculous mycobacteria (NTM). It can also be a secondary manifestation if it results from spreading bacteria from the main organ. Secondary TB manifestations are the most common mechanisms that occur. The spread of bacteria can be through the bloodstream or lymph vessels and then manifest in the lymph nodes, where inactive macrophages phagocytose the bacteria. The bacteria then replicate within the macrophages until the proteolytic enzymes and cytokines from the macrophages cause cell death and release of the bacteria. Monocytes and activated macrophages will form granulomas to limit bacterial infection, the mechanism can continue, and granulomas turn into lesions with necrosis in the center and the edges of fibrous tissue. TB lymphadenitis can also reactivate latent TB and can be caused by direct spread to the oropharyngeal mucosa in children (Rodriguez-Takeuchi *et al*., 2019; Gopalaswamy *et al*., 2021).

Cervical lymphadenitis is the most common form of tuberculous lymphadenitis; however, it can also involve lymph vessels throughout the body. Cervical lymphadenitis is the most common manifestation in children with good immune systems. The clinical manifestations are not so clear that it is challenging to distinguish TB Lymphadenitis from mild inflammatory conditions or other infections. The clinical picture is generally in the form of dense enlarged lymph nodes, sometimes accompanied by abscesses, and can cause sinuses, although it is scarce. Generally, enlargement of the gland is unilateral, single, and located on the right side of the cervix with a size of between 3-6 cm, and in some cases, there are lesions in the lungs (Chang *et al*., 2013; Zimmermann *et al*., 2015; Arbind *et al*., 2016; Gautam *et al*., 2018).

Diagnostic confirmation of suspected TB lymphadenitis using FNAB aspirate tissue specimen examination or biopsy, microscopic methods for detecting acid-fast bacteria (AFB), histopathology, culture, and molecular examination using polymerase chain reaction (PCR) techniques, as well as GeneXpert MTB/RIF. PCR examination is a complementary examination for TB lymphadenitis besides histopathology. It has been applied to detect Mycobacterium DNA sequences in various examination materials, one of which comes from aspirate from Fine Needle Aspiration Biopsy (FNAB) (Chakravorty *et al.*, 2005; Ligthelm *et al*., 2011; Coetzee *et al*., 2014).
In the treatment of TB Lymphadenitis, there are also difficulties. In a study, it was reported that there was a recurrence after treatment in TB Lymphadenitis patients after 28 months. As many as 3.8% of patients experienced a recurrence seen from the clinical picture, namely the presence of enlarged glands. The cause of recurrence after treatment is unknown, but examinations showed no drug resistance (Ko *et al*., 2019).

This study used the gyrB *Mycobacterium tuberculosis* complex (MTBC) gene as a primer based on its role in the metabolic process of MTBC bacterial cell replication. DNA gyrase in MTBC bacterial cells is a hetero tetramer (A2B2) molecule consisting of *gyrA* and *gyrB*. It plays a role in maintaining DNA topology, *gyrB*, in particular, initiates ATP hydrolysis (Kashyap *et al.*, 2018). Using ATP as a cofactor, gyrase controls the topology of the DNA by adding or removing supercoiled DNA. Gyrase facilitates DNA unwinding at the replication fork by introducing negative supercoils (Petrella *et al*., 2019). This process is related to the active replication of MTBC, giving rise to inflammatory manifestations in patients, namely swollen lymph nodes. The *gyrB* gene has a specific and conserved region in MTBC as a target for DNA amplification in the PCR method in this study. In the active replication of MTBC in tissue specimens of FNAB Lymphadenitis TB, it can be assumed that high levels of the gyrB gene are detected. With the PCR method, it can have high sensitivity and specificity.

Materials and Methods

Sample collection

The research sample was obtained from FNAB Lymphadenitis aspirate in patients with suspected Lymphadenitis TB, at Dr. Soetomo Academic Hospital Surabaya, from August to November 2020. The samples were examined histopathologically and Xpert MTB/RIF.

PCR assay

DNA extraction is conducted due to procedure guidelines in the DNeasy Blood & Tissue Kit [Qiagen, Jerman].
DNA amplification using the PCR method. A positive control using *M. tuberculosis* H37Rv. Identification of the MTBC group utilizing a pair of primers to amplify the target gyrB gene with the strand lengths presented in Table 1.

Table 1: Primer sequences used in the PCR process

Gene	Primer	Sequences (5'-3')	Size (bp)
gyrB	MTUB F	TCGGACGCGTATGCGATATC	1020
	MTUB R	ACATACAGTTCGGACTTGCG	

The amplified product was analyzed using electrophoresis with agarose gel. The gel was then placed on a UV transilluminator and documented using a digital camera (Sinha *et al*., 2016).

Histopathological examination

The examination was performed using the smear method with hematoxylin-eosin (HE) staining. Observation of preparations using an Olympus microscope with a magnification of 200x.

Ethical Considerations

This study received ethical approval, with certificate number 1636/KEPK/XI/2019 dated 9 November 2019, from the Health Research Ethics Committee of Dr. Soetomo Academic Hospital Surabaya.

Results

Total suspected Lymphadenitis TB were 39 samples from August 2020 to November 2020, and 8 positive Xpert MTB/RIF (20.5%). In this study, 15 samples were subjected to molecular and histopathological examinations.

PCR assay of *gyrB* gene from FNAB aspirate of lymphadenitis

The aspirate from FNAB was subjected to molecular examination using the gyrB - PCR method using the target *Mycobacterium tuberculosis* gryB gene with a sequence length of 1020 bp. The results of this study indicate that of the 15 aspirate samples, 6 (40%) of them showed positive results.

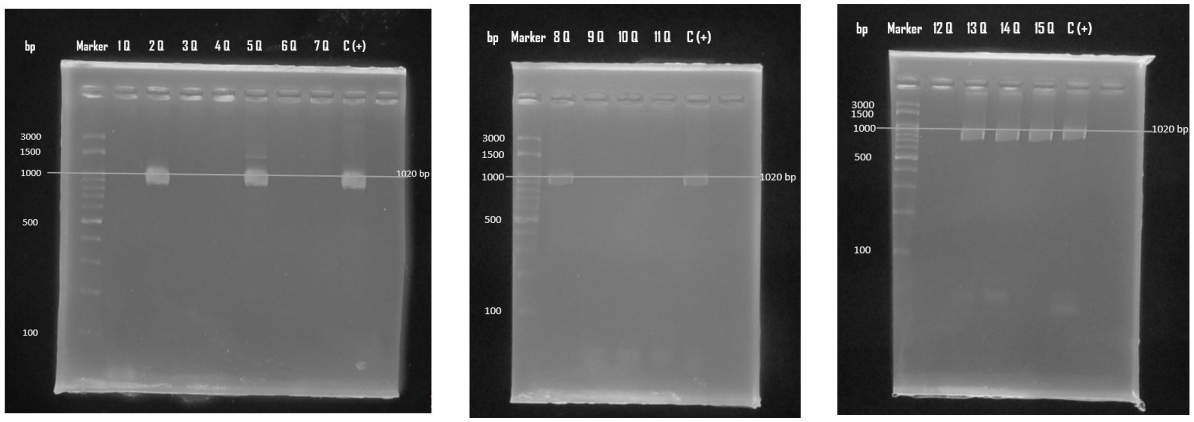
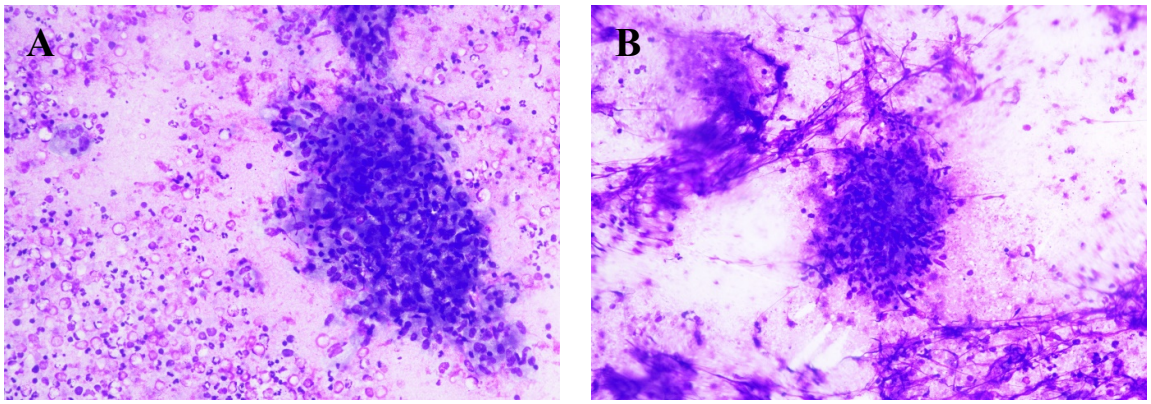


Figure 1:PCR examination results for detecting the gyrB gene for *M. tuberculosis* FNAB Lymphadenitis aspirate sample. The figure showed the 1020 bp band on the C(+) control of the *M. tuberculosis* H37Rv strain; note the positive band on samples 2, 5, 8, 13, 14, and 15.

From Figure 1, it is obvious that samples 2, 5, 8, 13, 14, and 15 showed the same 1020 bp DNA band (band) as the positive control band of *Mycobacterium tuberculosis* H37Rv. This stated that the six samples were positive for *Mycobacterium tuberculosis*.

Histopathological examination analysis



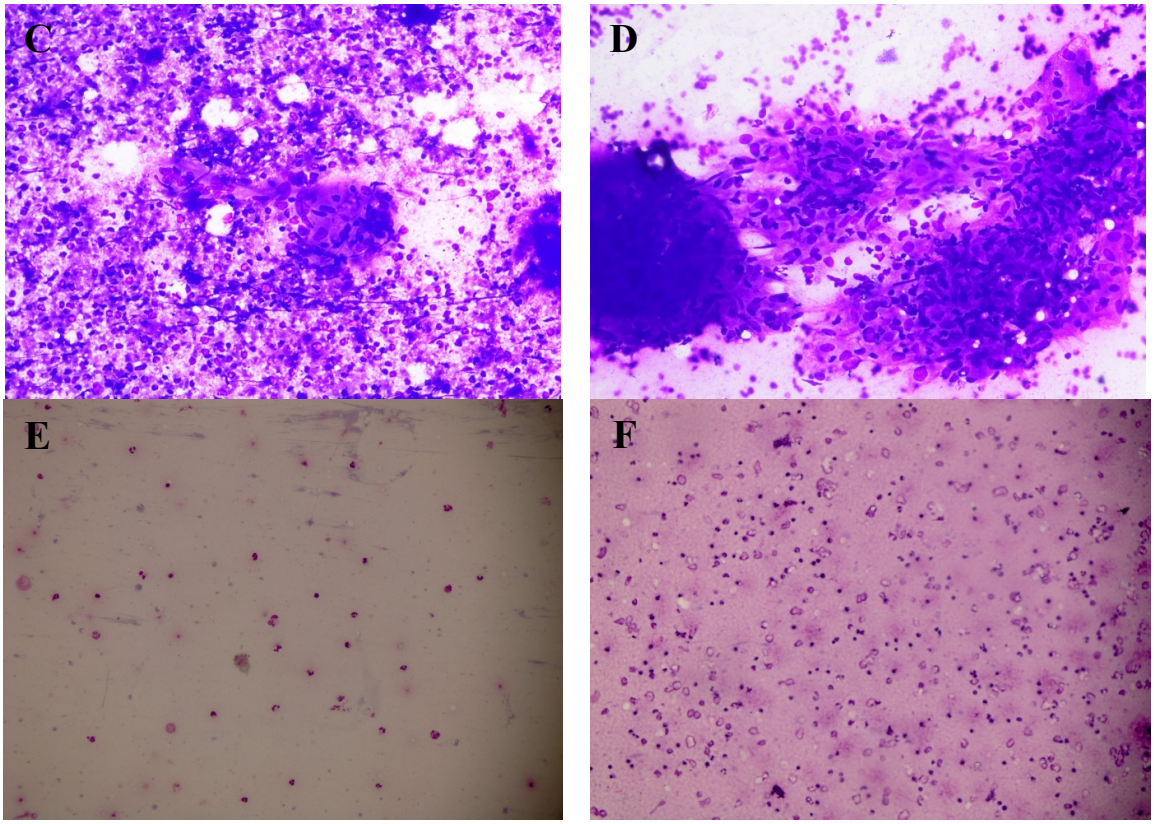


Figure 2: Histopathological examination of the FNAB aspirate showed positive results in images A-D. Images E and F show a negative result (200x Mag.)

The results of the histopathological examination of the molecularly positive samples are shown in Figure 2. Histopathologic characteristic features are positive for 4 of 15 samples (26.66%). In specimens that showed positive histopathological characteristics of tuberculosis, it was seen that the cell smear contained groups of epitheloid cells that formed granulomas against a background of lymphoid cells and a dense distribution of PMN inflammatory cells, as well as necrotic debris. Also seen are multinucleated giant cells, PMN cells, and amorphous debris. This suggests chronic granulomatous inflammation consistent with tuberculosis. In samples that showed a negative histopathological appearance, lymphocytes, PMNs and lymphoid cells of various maturities were distributed. No granuloma characteristic features were found, with the conclusion of chronic suppurative inflammation.

Table 2: FNAB aspirate specimens from patients with suspected TB lymphadenitis based on the results of histopathological examination, MTBC detection (gyrB-gene).

	***M. tuberculosis* (+)**	***M. tuberculosis* (-)**
Histopatology (+)	4	0
Histopatology (-)	2	9

Discussion

In this study, 15 lymphadenitis FNAB aspirate samples were collected from patients, and six were positive for *Mycobacterium tuberculosis* PCR gyrB. In the other nine samples, MTBC was not found based on gyrB - PCR examination. In patients with negative gyrB - PCR test results, the lymphadenitis may be caused by NTM infection, other microbes, or malignancy (Willemse *et al*., 2018; Sarfaraz *et al*., 2018). Specific and conserved gyrB gene regions of MTBC detection can be stated as highly specific.

The paucibacillary nature of TB Lymphadenitis specimens makes diagnosis difficult and requires a combination of clinical, radiological, microbiological, and molecular examinations. Combined cytology characteristics

(positive for epithelioid cell granuloma, multinucleated giant cells, and granuloma lesions with caseous necrosis) (Anggraini and Oktora, 2021; Djannah *et al*., 2022).

In FNAB, the aspirate material taken can be used for cytology testing, acid-fast bacilli (AFB) staining, culture, and molecular examination. FNAB cytology showed the formation of epithelioid granulomas and caseous necrosis. Such findings suggest a tubercle etiology, especially in developing countries with high TB. The sensitivity and specificity of FNAB cytology for the diagnosis of lymphadenitis were 88% and 96%. However, granulomas and caseous are rare in HIV-positive TB lymphadenitis due to impaired T-cell function. The combination of FNAB cytology and culture or rapid molecular test increases the diagnostic value of TB cervical lymphadenitis (Kudu *et al*., 2020; Minnies *et al*., 2021).

The detection rate of *M. tuberculosis* in FNAB aspirates is relatively low by microbiological techniques. The positive value ranges from 15% - 47%, depending on the presence or absence of necrosis in patients with a history of TB. Cultures of the aspirate have been reported in some studies to be positive in 35% to 65%. In some cases, specimens were also found that were positive for TB on culture examination but negative on PCR examination, even after repeated trials. This is caused by very low levels of bacteria that cause false negatives on molecular analysis (Gautam *et al*., 2018; Singh, 2000).

In general, the concentration of DNA from the aspirate sample is low due to its paucibacillary nature. Inaccurate sampling techniques can also cause low DNA concentrations. The FNAB performed may need to be more precise on the granuloma so that bacteria are not found or only in very low numbers. This problem can be solved by using computed tomography (CT) guidance. The positivity rate of using CT guiding on FNAB examination was 95.4% in lymphadenitis patients (Kiral *et al*., 2015). In this case, CT guiding must be carried out as a mandatory procedure to improve the quality of diagnosis of TB Lymphadenitis patients.

Conclusion

In this study, six samples (40%) positive of 15 FNAB samples of suspected Lymphadenitis TB patients were detected in the diagnostic based on molecular detection using a specific and conserved gene target, gyrB – PCR. Characteristic features of the histopathological examination in conjunction with positive gyrB - PCR in the FNAB of the Lymphadenitis aspirate sample can be used to determine Lymphadenitis TB.

Acknowledgements

We want to thank the Master of Tropical Medicine Study Program, Faculty of Medicine, Airlangga University, Clinical Microbiology and Anatomical Pathology Installation at Dr. Soetomo Hospital, Surabaya, Indonesia, and the Tuberculosis Laboratory, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest whatsoever associated with this study.

References

1. Anggraini, D., and Oktora, M. Z. (2021). “Hematology Profile of Tuberculosis Lymphadenitis Patients at Siti Rahmah Hospital, Padang, Indonesia.” *Indonesian Journal of Clinical Pathology and Medical Laboratory;* 27 (3): 271–75.
2. Arbind, A., D′souza, M., Jaimini, A., Saw, S., Solanki, Y., and Sharma, R. (2016). “Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Imaging in Response Monitoring of Extrapulmonary Tuberculosis.” *Indian Journal of Nuclear Medicine;* 31 (1): 59.
3. Baykan, A. H., Sayiner, H. S., Aydin, E., Koc, M., Inan, I., and Erturk, S. M. (2022). “Extrapulmonary Tuberculosıs: An Old but Resurgent Problem.” *Insights into Imaging;* 13 (1): 39.
4. Chakravorty, S., Sen, M. K., and Tyagi, J. S. (2005). “Diagnosis of Extrapulmonary Tuberculosis by Smear, Culture, and PCR Using Universal Sample Processing Technology.” *Journal of Clinical Microbiology;* 43 (9): 4357–62.
5. Chang, C. Y., Hong, J. Y., Yuan, M. K., Chang, S. J., Lee, Y. M., Chang, S. C., Hsu, L. C., and Cheng, S. L. (2013). Risk factors in patients with AFB smear-positive sputum who receive inappropriate antituberculous treatment. *Drug design, development and therapy*; 7, 53–58.
6. Coetzee, L., Nicol, M. P., Jacobson, R., Schubert, P. T., van Helden, P. D., Warren, R. M., and Wright, C. A. (2014). “Rapid Diagnosis of Pediatric Mycobacterial Lymphadenitis Using Fine Needle Aspiration Biopsy.” *Pediatric Infectious Disease Journal;* 33 (9): 893–96.
7. Djannah, F., Massi, M. N., Hatta, M., Bukhari, A., and Hasanah, I. (2022). “Profile and Histopathology Features of

Top Three Cases of Extra Pulmonary Tuberculosis (EPTB) in West Nusa Tenggara: A Retrospective Cross-Sectional Study." *Annals of Medicine and Surgery;* 75 (March): 103318.
8. Gautam, H., Agrawal, SK., Verma, SK., and Singh, UB. (2018). "Cervical Tuberculous Lymphadenitis: Clinical Profile and Diagnostic Modalities." *International Journal of Mycobacteriology;* 7 (3): 212.
9. Gopalaswamy, R., Dusthackeer, V. N. A., Kannayan, S., and Subbian, S. (2021). "Extrapulmonary Tuberculosis—An Update on the Diagnosis, Treatment and Drug Resistance." *Journal of Respiration;* 1 (2): 141–64.
10. Kashyap, A., Singh, P. K., and Silakari, O. (2018). "Chemical Classes Targeting Energy Supplying GyrB Domain of Mycobacterium Tuberculosis." *Tuberculosis;* 113 (December): 43–54.
11. Kiral, N., Caglayan, B., Salepci, B., Parmaksiz, E. T., Fidan, A., Comert, S. S., Yavuzer, D., and Partal, M. (2015). "Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration in Diagnosing Intrathoracic Tuberculous Lymphadenitis." *Medical Ultrasonography;* 17 (3): 333.
12. Ko, Y., Kim, C., Park, Y., Mo, E. K., Moon, J. W., Park, S., Sim, Y., Hong, J., and Baek, M. (2019). "Clinical Characteristics and Treatment Outcomes of Definitive versus Standard Anti-Tuberculosis Therapy in Patients with Tuberculous Lymphadenitis." *Journal of Clinical Medicine;* 8 (6): 813.
13. Kudu, A. T. D., Iliya, S., Atanda, A. T., Ismail, N. A., and Shehe, A. A. (2020). "Diagnosis of Extrapulmonary Tuberculosis by MTB/RIF from a Fine Needle Aspirate Biopsy: Case Report." *Journal of Microbiology and Infectious Diseases*; 10(1) 59–61.
14. Ligthelm, L. J., Nicol, M. P., Hoek, K. G. P., Jacobson, R., van Helden, P. D., Marais, B. J., Warren, R. M., and Wright, C. A. (2011). "Xpert MTB/RIF for Rapid Diagnosis of Tuberculous Lymphadenitis from Fine-Needle-Aspiration Biopsy Specimens." *Journal of Clinical Microbiology;* 49 (11): 3967–70.
15. Minnies, S., Reeve, B. W. P. Rockman, L., Nyawo, G., Naidoo, C. C., Kitchin, N.,and Rautenbach, C. (2021). "Xpert MTB/RIF Ultra Is Highly Sensitive for the Diagnosis of Tuberculosis Lymphadenitis in a High-HIV Setting." Edited by Christine Y. Turenne. *Journal of Clinical Microbiology;* 59 (12): 1-11.
16. Petrella, S., Capton, E., Raynal, B., Giffard, C., Thureau, A., Bonneté, F., Alzari, P. M., Aubry, A., and Mayer, C. (2019). "Overall Structures of Mycobacterium Tuberculosis DNA Gyrase Reveal the Role of a Corynebacteriales GyrB-Specific Insert in ATPase Activity." *Structure;* 27 (4): 579-589.e5.
17. Rodriguez-Takeuchi, Yukie, S., Renjifo, M. E., and Medina, F. J. (2019). "Extrapulmonary Tuberculosis: Pathophysiology and Imaging Findings." *RadioGraphics;* 39 (7): 2023–37.
18. Sarfaraz, S., Iftikhar, S., Memon, Y., Zahir, N., Hereker, F. F., and Salahuddin, N. (2018). "Histopathological and Microbiological Findings and Diagnostic Performance of GeneXpert in Clinically Suspected Tuberculous Lymphadenitis." *International Journal of Infectious Diseases;* 76 (1): 73–81.
19. Singh, K. K. (2000). "Comparison of in House Polymerase Chain Reaction with Conventional Techniques for the Detection of Mycobacterium Tuberculosis DNA in Granulomatous Lymphadenopathy." *Journal of Clinical Pathology;* 53 (5): 355–61.
20. Sinha, P., Gupta, A., Prakash, P., Anupurba, S., Tripathi, R., and Srivastava, G. N. (2016). "Differentiation of Mycobacterium Tuberculosis Complex from Non-Tubercular Mycobacteria by Nested Multiplex PCR Targeting IS6110, MTP40 and 32kD Alpha Antigen Encoding Gene Fragments." *BMC Infectious Diseases;* 16 (1): 123.
21. Soekotjo, F. N., Sudarwati, S., and Alam, A. (2019). "Clinical Profile of TB in Children at Pediatric Outpatient Clinic Hasan Sadikin Hospital Bandung 2016." *Journal of Medicine & Health;* 2 (3): 818-827
22. Willemse, S. H., Oomens, M. A. E. M., De Lange, J., and Karssemakers, L. H. E. (2018). "Diagnosing Non-tuberculous Mycobacterial Cervicofacial Lymphadenitis in Children: A Systematic Review." *International Journal of Pediatric Otorhinolaryngology;* 112 (1): 48–54
23. World Health Organization. (2021). *Global Tuberculosis Report 2021.* https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2021 Accessed 24 May 2023
24. Zimmermann, P., Tebruegge, M., Curtis, N., and Ritz, N. (2015). "The Management of Non-Tuberculous Cervicofacial Lymphadenitis in Children: A Systematic Review and Meta-Analysis." *Journal of Infection;* 71(1): 9–18.