Available online at IJTID Website: https://e-journal.unair.ac.id/IJTID/

Indonesian Journal of
Tropical and Infectious Disease

Vol. 9 No. 1 January–April 2021

Original Article

Diagnosis Based on Detection of CXCL10 in Urine as Biomarker for The Determining Diagnosis of Active Lung Tuberculosis

I Gede Yogi Prema Ananda[1], Ni Made Mertaniasih[2]*, Soedarsono[3], Deby Kusumaningrum[2]

[1]Faculty of Medicine Universitas Airlangga, Surabaya Indonesia

[2] Department of Medical Microbiology, Faculty of Medicine Universitas Airlangga, Surabaya Indonesia

[3] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

Received: 24th September 2020; Revised: 4th February 2019; Accepted: 9th February 2021

ABSTRACT

Tuberculosis diagnosis is an important component in decreasing TB incidence and prevalence. Because of the difficulty to collect sputum in some cases, urine specimens are used as it is easier to garner. One of the biomarkers in urine that can be used to diagnose pulmonary TB is IP-10, which can be represented by the CXCL10 gene. The study aims to determine the accuracy of diagnosis based on detection of the CXCL10 gene in urine as a biomarker for the patients with suspected pulmonary TB in Dr. Soetomo Hospital in Surabaya from November 2019 until March 2020. Thus, this is an observative laboratory research with a cross-sectional study. CXCL10 gene was examined using PCR for 36 urine samples, and then, the data, together with the medical records of clinical manifestations of pulmonary TB, GeneXpert MTB /RIF, blood count, and thorax radiograph, were processed using IBM SPSS Statistics 26. The results of the GeneXpert MTB/RIF and thorax radiograph criteria show positive results of pulmonary TB, which were 44.4% and 69.4% respectively. CXCL10 gene was not found in all urine of healthy people (negative), while 2.8% (1/36 samples) positive CXCL10 gene was found in a patient with positive GeneXpert, also with negative clinical manifestations and urine culture. In this study, the accuracy of diagnosis based on detection of the CXCL10 gene in urine for diagnosis of active pulmonary TB was 2.8%. Future research is needed to improve the methods, among them are bigger size of urine samples and clearer medical history of patients.

Keywords: *Tuberculosis; CXCL10; Biomarker; Urine; Diagnosis*

ABSTRAK

Diagnosis tuberkulosis merupakan komponen penting dalam menurunkan insiden dan prevalensi TB. Karena sulitnya mengumpulkan dahak pada beberapa kasus, spesimen urin digunakan karena lebih mudah didapatkan. Salah satu biomarker dalam urin yang dapat digunakan untuk mendiagnosis TB paru adalah IP-10 dengan cara mendeteksi keberadaan gen CXCL10. Penelitian ini bertujuan untuk mengetahui akurasi diagnosis berdasarkan deteksi gen CXCL10 dalam urin sebagai biomarker untuk diagnosis pasien TB Paru di RSUD Dr. Soetomo Surabaya dari November 2019 hingga Maret 2020. Oleh karena itu, penelitian ini termasuk penelitian laboratorium observatif dengan studi cross-sectional. Pemeriksaan gen CXCL10 dilakukan menggunakan PCR, kemudian data, bersama dengan hasil rekam medis manifestasi klinis TB paru, GeneXpert MTB/RIF, menghitung darah, dan rontgen dada, diolah menggunakan IBM SPSS Statistics 26. Hasil kriteria GeneXpert MTB/RIF dan rontgen dada menunjukkan hasil positif masing-masing 44,4% dan 69,4% TB paru. Semua urine orang sehat menunjukkan hasil gen CXCL10 negatif, didapatkan hasil sebesar 2,8% gen CXCL10 positif dalam urin pasien dengan GeneXpert positif dengan manifestasi klinis dan kultur urin negatif. Dalam penelitian ini akurasi diagnosis berdasarkan deteksi gen CXCL10 dalam urin untuk diagnosis TB paru aktif adalah 2,8%. Penelitian lebih lanjut dibutuhkan untuk meningkatkan metode yang digunakan, terutama agar menggunakan lebih banyak sampel urin dan riwayat pasien yang lebih jelas.

* Corresponding Author:
nmademertaniasih@gmail.com

Kata kunci: *Tuberkulosis; CXCL10; Biomarker; Urin; Diagnosis*

How to Cite: Ananda, IGYP., Mertaniasih, NM., Soedarsono., Kusumaningrum, D.Diagnosis Based on Detection of CXCL10 in Urine as Biomarker for The Determining Diagnosis of Active Lung Tuberculosis. Indonesian Journal of Tropical and Infectious Disease, 9(1), 57–65.

INTRODUCTION

Tuberculosis (TB) is a pulmonary infectious disease caused by Mycobacterium tuberculosis and one of the top 10 causes of death as well as the number one cause of death from infection in the world. In 2017, 1.3 million people died from TB, and 10 million people were infected with TB.[1] Again, in 2018, as many as 1.3 million people died from TB and 10 million people were infected.[2] Indonesia is one of the 20 countries with the most TB cases, with 845 thousand people infected and 563 thousand diagnosed, and 98 thousand of them died from TB in 2018. Indonesia is included in High Burden Countries (HBC), countries with a high burden of TB based on 3 indicators, namely TB, HIV-TB coinfection, and MDR-TB. As Indonesia is included in all indicators, TB becomes one of the main health problems in Indonesia.[2]

In Indonesia, the detected and reported TB cases were 53% in 2017.[1] And then, it increased to 67% in 2018.[2] Of the unreported cases, 29% were detected but not reported and 18% were not detected at all. Java and Bali are the regions with the highest number of unreported TB cases, which is at 42%. Puskesmas as primary care in Indonesia has 15% of unreported cases, relatively lower than cases not reported by hospitals, which reaches 65%, and the highest by a combination of general practitioners, clinics, and laboratory practices was 96%.[1] Indonesia targets to increase TB disease control by decreasing the number of people with TB disease from 293 people per 100,000 population in 2013 to 245 people per 100,000 population in 2019 (4). Indonesia also targets TB elimination by 2035 and TB-free Indonesia by 2050.[5]

Diagnosis is an important component in achieving the target of reducing TB incidence and prevalence. Diagnosis of pulmonary tuberculosis begins with clinical criteria, chronic cough symptoms for more than 2 weeks, accompanied by fever, night sweats, and weight loss. For a country with a high TB prevalence such as Indonesia, all patients with suspected pulmonary TB clinical criteria are immediately diagnosed with pulmonary TB disease. The problem is that not all patients showing symptoms of chronic cough proved to be Acid Resistant Basil (AFB) positive, and vice versa. Data shows that 10-25% of patients with positive smear do not show symptoms of cough.[3]

The most common laboratory microscopic examinations are the sputum smear using the Ziehl-Neelsen staining technique and the GeneXpert MTB/RIF Molecular Rapid Test.[3] Both of these methods have disadvantages, that it is difficult for the patient to pass sputum, and consequently, there have not been enough sputum specimens collected for examination. A method of examination using specimens that is easier to collect, such as urine, is needed. It is easier to ask the patient to urinate than to expel phlegm. Besides, urine collection is non-invasive, not too risky for the medical personnel involved, and requires no special equipment or expertise.

One of the biomarkers in urine that can be used for diagnosis of pulmonary TB is IP-10, or Interferon-gamma(IFN-γ)-inducible protein of 10 kDa, which is represented by the CXCL10 gene, a pro-inflammatory chemokine released by exposed cells with antigens and cause activated *T lymphocytes* to move toward

the site of inflammation. Inflammation in active pulmonary TB spreads inflammatory cells lymphogenously and haematogenously throughout the body, including the kidneys, to be excreted together with urine. Urinary IP-10 levels are significantly elevated in patients with pulmonary disease, be it TB or other infections. The level of IP-10 in the urine of pulmonary TB patients who were examined at the onset of the disease was higher than in patients who had recovered.[12] (Cannas et al., 2010). IP-10 levels increase in patients with active pulmonary TB and decrease when TB treatment is complete.[19] This study aims to analyze the accuracy of the diagnosis based on the detection of the CXCL10 gene in the urine of patients with suspected pulmonary TB. The results of this study are expected to determine the accuracy of the CXCL10 gene detection method in urine as a laboratory tool for diagnosis of pulmonary TB.

MATERIALS AND METHODS

This research was a laboratory observational study with a cross-sectional study design using primary data of the results of the CXCL10 gene examination using PCR and secondary data of medical records unit in Dr. Soetomo Hospital. Medical records include clinical manifestations of pulmonary tuberculosis, Molecular Rapid Test of GeneXpert MTB/RIF (Cepheid, Canada), laboratory examinations, complete blood count, physical examination, and radiological photos of the thorax. The research was conducted in the period of November 2019 - March 2020. Urine samples were collected from 36 pulmonary TB adult patients. Laboratory procedure for urine examination was urine processing using centrifugation, DNA extraction using TE buffer with boiling, and PCR optimization. The primer for PCR were 5'-TTCCTGCAAGCCAATTTTGTCC-3' for forward and 5'- for the reverse. GCAGCTGATTTGGTGACCAT-3' 3 urine Urine culture based on standard solid medium culture method, 200 µL sediment of urine processing, was inoculated in Middlebrook 7H10. The accuracy was determined by detecting the CXCL10 gene, which represented IP-10 protein in the urine of active pulmonary TB patients, and Nucleic Acid Amplification Tests (NAATs) method using PCR. The results was determined as positive if the band measured was 305 basepair (bp) and it matched with the primer set. The collected data were then processed with IBM SPSS Statistics 26. After processing the data, the next step was to analyze the data whether the existing research hypothesis were to be accepted or rejected. Data analysis was used to describe, understand, and explain the relationship between the variables studied.

RESULTS

Based on the study, there were 36 samples of patients with suspected pulmonary TB consisting of 20 (55.6%) men and 16 (44.4%) women. Most samples were found in the age range of 20-29 years old, i.e., 10 people (27.8%). The complete findings are: 4 people aged 10-19 (11.1%), 1 person aged 30-39 (2.8%), 6 people aged 40-49 (16.7%), 3 people aged 50-59 (8.3%), 8 people aged 60-69 (22.2%), and 4 people aged 70-79 (11, 1%). The results shows that there were 33.3% of patients with low BMI, 2.8% of patients with high BMI, and 63.9% of patients with normal BMI.

The majority of the research samples came from Surabaya with 20 people (55.6%), while 16 people (44.4%) came from outside Surabaya. The majority of the sample, 15 people (41.7%) of the 36, did not work, 10 of them were private workers (28.7%), 6 were students or students (16.7%), 3 were farmers (8.3%), while for merchants and regional senators (DPRD) were with the same number, each consisting of 1 person (2.8%) as can be seen in Table 1.

Table 1. Frequency distribution of patients with lung TB suspect based on characteristics in the DOTS clinic of Dr. Soetomo Surabaya in November 2019 - March 2020 period

Characteristics	Total	Percentage
Gender		
Male	20	55,6%
Female	16	44,4%
Total	36	100%
Age		
10-19	4	11,1%
20-29	10	27,8%
30-39	1	2,8%
40-49	6	16,7%
50-59	3	8,3%
60-69	8	22,2%
70-79	4	11,1%
Total	36	100%
Body Mass Index		
Normal	23	63,9%
Underweight	12	33,3%
Overweight	1	2,8%
Region		
Surabaya	20	55,6%
Outside Surabaya	16	44,4%
Total	36	100%
Job		
Student	6	16,7%
Private Worker	10	27,8%
Farmer	3	8,3%
Merchant	1	2,8%
DPRD	1	2,8%
No Job	15	41,7%
Total	36	100%

The comparison of the results of CXCL10 gene detection in urine with clinical manifestations has a specificity of 100%, a sensitivity of 6.6%, and an accuracy of 61.1% as can be seen in Table 2.

Table 2. Comparison of the results of the CXCL10 gene detection in urine with clinical manifestations

Detection of the CXCL10 Gene in Urine		Clinical Manifestation		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	14	21	35
	%	38,90%	58,30%	97,20%
Total	Total	15	21	36
	%	41,70%	58,30%	100,00%

The comparison of the results of the CXCL10 gene detection in urine with physical examination has a specificity of 97.2% and sensitivity of 2,8% as can be seen in Table 3.

Table 3. Comparison of the results of the CXCL10 gene detection in urine with physical examination

Detection of the CXCL10 Gene in Urine		Physical Examination		Total
		Positive	Negative	
Positive	Total	0	1	1
	%	0,00%	2,80%	2,80%
Negative	Total	0	35	35
	%	0,00%	97,20%	97,20%
Total	Total	0	36	36
	%	0,00%	100,00%	100,00%

The comparison of the results of the CXCL gene detection in urine with the manifestations of laboratory tests of complete blood has a specificity of 100%, sensitivity of 11%, and accuracy of 77.7% as can be seen in Table 4.

Table 4. Comparison of CXCL10 gene detection results in urine with manifestations of complete blood count

Detection of The CXCL10 Gene in Urine		Complete Blood Count		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	8	27	35
	%	22,20%	75,00%	97,20%
Total	Total	9	27	36
	%	25,00%	75,00%	100,00%

The comparison of the results of the CXCL gene detection in urine with the radiological results of the chest radiograph has a specificity of 100%, sensitivity of 4% and an accuracy of 33.3% as can be seen in Table 5.

Table 5. Comparison of the results of the CXCL gene detection in urine with the results of cadiological chest radiographs

Detection of the CXCL10 Gene in Urine		Chest Radiograph		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	24	11	35
	%	66,70%	30,50%	97,20%
Total	Total	25	11	36
	%	69,50%	30,50%	100,00%

The comparison of the results of the CXCL gene detection in urine with the results of GeneXpert has a specificity of 100%, a sensitivity of 6.2%, and an accuracy of 58.3% as can be seen in Table 6.

Table 6. Comparison of CXCL10 gene detection results in urine with GeneXpert results

Detection of the CXCL10 Gene in Urine		GeneXpert		Total
		Positive	Negative	
Positive	Total	1	0	1
	%	2,80%	0,00%	2,80%
Negative	Total	15	20	35
	%	41,70%	55,60%	97,20%
Total	Total	16	20	36
	%	44,40%	55,60%	100,00%

The comparison of the results of the CXCL gene detection in urine with the results of urine culture has a specificity of 97.2%, a sensitivity of 0%, and an accuracy of 97.2% as can be seen in Table 7.

Table 7. Comparison of the Results of the CXCL gene detection in urine with the results of urine culture

Detection of the CXCL10 Gene in Urine		Urine Culture Result		Total
		Positive	Negative	
Positive	Total	0	1	1
	%	0,00%	2,80%	2,80%
Negative	Total	0	35	35
	%	0,00%	97,20%	97,20%
Total	Total	0	36	36
	%	0,00%	100,00%	100,00%

patients (10.8%) with MRSA carrier events as much as zero (0%).[18]

DISCUSSION

In this study, the prevalence of MRSA in subjects with stage five CKD were 6/150 (4%) there were no significant differences in the incidence of MRSA carriers in stage five CKD non HD or HD groups. This study shows that MRSA colonization exists in stage five CKD sufferers who have or who have not received HD therapy.

Pulmonary tuberculosis is a disease caused by Mycobacterium tuberculosis. These bacteria are transmitted through droplets that enter the respiratory tract. The clinical symptoms are 3 weeks or more cough with phlegm, hemoptysis, fever, chest pain, weight loss, night sweats, and tightness. The results show that 91.7% of the patients had cough symptoms for 3 weeks or more. This is supported by a research conducted.[8] which states that 81% of patients had cough symptoms for 3 weeks or more

Low BMI is associated with the risk of developing pulmonary TB because it is correlated with malnutrition and susceptibility to infectious diseases. The results show that there were 33.3% of patients with low BMI, 2.8% of patients with high BMI, and 63.9% of patients with normal BMI. Research states that low BMI correlates with the risk of being infected with pulmonary TB, but not with extrapulmonary TB.[13] Research in Taiwan also states that low BMI increases the risk of infection and mortality of TB disease.[28] Another study in Korea shows that a high BMI lowers the risk of TB infection, but a very high BMI does not reduce the risk.[20] Meanwhile, research in China shows that high BMI and obesity are associated with the risk of TB infection, possibly because the excess cell adiposity weakens the immune system.[30]

The majority of patients showed that vital signs were outside the normal limits, and the results of the study showed that most of the patients had abnormal temperatures, which was in 33.3% of patients. Another study states

that 22.5% of 40 patients experienced increased body temperature as a result of TB disease.[24] The vital sign that was most often outside the normal limit was the respiratory rate, with 79.5% of 49 patients showing a respiratory rate that exceeded the normal limit.[26]

Complete blood count can be a parameter for diagnosis, prognosis, or response to pulmonary TB disease treatment.[27] The results show that 19.4% of patients had leukocytosis and 5.6% of patients had decreased hemoglobin (Hb). TB patients experienced decreased leukocytosis and Hb. Decreased hemoglobin is one of the hematological problems that often appears in TB patients.[23] There were 61.5% of TB patients with anemia, where 43% of them suffered from moderate and severe anemia, and 49% suffered from iron deficiency anemia and anemia of chronic diseases.[9]

A chest radiograph is an important examination for people with suspected pulmonary tuberculosis but showing negative results of smear examination. It can also be used to determine disease progression and evaluate responses to therapy. Radiological photos alone cannot diagnose pulmonary tuberculosis, it is also necessary to combine it with a physical examination and clinical symptoms.[3] The results show that there were 69.4% of patients with positive chest radiology results. In the right lung, the most found were infiltrates and infiltrates accompanied by fibrosis, each of 5 people (13.9%), pleural effusions as many as 4 people (11.1%), and cavities accompanied by infiltrates as many as 2 people (5,6 %). In the left lung, the most found was infiltrates as many as 5 people (13.9%) and infiltrates with fibrosis in 3 people (8.3%). The most common features on chest radiological radiographs include 55% consolidation, 26% pleural effusion, and 17% lung collapse (Appleton et al., 2017). Another study shows that the most commonly seen features were 45% non-specific apex, 33% normal apex, and 16% apex of the lung with infiltrates. When compared with patients whose culture results were negative, pulmonary TB patients showed 43% of infiltrates at the apex and 14% of cavities.[6] Pleural effusion was found in 38% of new cases of pulmonary TB patients.[10]

Research conducted in Nigeria shows that the use of GeneXpert for the diagnosis of pulmonary tuberculosis has better results than smear testing.[15] The results show that there were 44.4% of patients with positive GeneXpert results, and 2.8% of patients showed resistance to Rifampicin. Examination using GeneXpert is more accurate than occasional sputum examination with higher sensitivity and negative predictive value (NPV).[29] Another study conducted in China shows that examination with GeneXpert has a sensitivity of 94.6%, specificity of 82.9%, positive predictive value (PPV) 77.3%, and negative predictive value (NPV) of 96.1% so that it can be used for examinations that require a shorter time, are simpler, and more efficient.[23]

Research conducted in Iran shows that 3.1% of 162 pulmonary TB patients whose sputum test results were positive were also resistant to Rifampicin from the results of the GeneXpert examination.[7] Another study conducted in Ethiopia shows that 1 out of 14 pulmonary TB patients who were bacteriologically positive was also resistant to Rifampicin.[17]

In this study, the results show that none of the patients had positive urine culture results. Meanwhile, a study conducted on HIV positive pulmonary TB patients in Ethiopia shows that urine culture could help improve detection of the bacterium *Mycobacterium tuberculosis* in HIV positive patients. Of the 45 people, there were 14.5% positive culture patients in Lowenstein-Jensen media, 6% positive culture patients from urine smears, and 24.8% positive patients from RD9-based PCR examinations.[14] Another study conducted in India shows that 26.1% of the 46 patients with suspected pulmonary TB with positive sputum culture results also showed positive urine

culture results.[16]

The results of this study show that there were 2.8% of patients with positive urine detection of the CXCL10 gene. The detection accuracy of the CXCL10 gene in urine was 2.8% compared with clinical manifestation of pulmonary TB, chest radiograph, and GeneXpert. Active pulmonary TB patients have higher urine levels of the CXCL10 gene than healthy people. The detection of CXCL10 in urine using ELISA (Quanterix) has a sensitivity of 78% and a specificity of 94%.[22] The detection of the CXCL10 gene in serum has a sensitivity of 87.5% and a specificity of 78.9%.[21] Another study reveals that detection of CXCL10 in serum showed positive results in 87.5% of active pulmonary TB patients, 45.5% of latent TB, and 9.5% in control variables.[18]

The differences in the accuracy rate between this research and other studies may be caused by some reasons. The study from Petrone et al. in 2019.[22] was conducted using ELISA (Quanterix) to measure CXCL10 in urine, while this research was using PCR. Studies by Nonghanphithak et al. in 2017. [21] and Hong et al. in 2012.[18] measured CXCL10 in serum of TB patient, while this research measured it in urine to avoid invasive procedures.

If the detection of the CXCL10 gene in urine is to be compared with other tests, the highest sensitivity is shown by complete blood laboratory examination, which is 11%. The examination with the highest specificity are shown in the manifestation of clinical symptoms, complete blood laboratory tests, gene GeneXpert results, and radiological radiographs of the chest, which are 100% respectively. The examination with the highest accuracy is physical examination and urine culture result, which is 97.2%.

This study has limitations in that the samples representing low BMI and optimal BMI are not sufficient. BMI is also a determining factor for the production of the CXCL10 gene. Also, it was difficult to determine the duration of clinical symptoms that varied among the patients.

Overall, the accuracy of the method of diagnosing pulmonary TB based on detection of the CXCL10 gene in urine cannot be measured because of several reasons, i.e., lack of medical history, especially in treatment of anti-tuberculosis drugs, small sample size of active TB patients, and the difficulty to collect urine directly from hospitalized patients. Further research in cohort study is needed with more complete clinical variable data, a wider scope, more samples, a real-time PCR method to detect the CXCL10 gene in urine, and the Next Generation Sequencing (NGS) method. The validity of this research can also be increased by using 50 ml of the patients' first morning urine.

CONCLUSION

In this study, the accuracy of diagnosis based on detection of the CXCL10 gene in urine as a biomarker for diagnosis of active pulmonary TB is 2.8%. Future research is needed to improve the methods by increasing urine samples to 50 ml and using clear medical history of the patients especially the history of anti-tuberculosis drugs.

CONFLICT OF INTEREST

There is no confict of interest regarding this study.

ACKNOWLEDGEMENT

The authors would like to thank Mrs. Agnes, Mrs. Ria, Mr. Amin, and Mr. Agus for the help as lab technicians, to Mrs. Sri and the other nurses for the help in getting samples, and to all the patients.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2018. *Geneva*: World Health Organization; 2018
2. World Health Organization. Global Tuberculosis Report 2019. *Geneva*: World Health Organization; 2019
3. World Health Organization. International Standards For Tuberculosis Care. *San Fransisco*: World Health Organization; 2014
4. Kementerian Kesehatan Republik Indonesia. Rencana Strategis Kementerian Kesehatan Tahun 2015-2019. Jakarta: Kementerian Kesehatan Republik Indonesia; 2015
5. Kementerian Kesehatan Republik Indonesia. Peraturan Menteri Kesehatan Republik Indonesia Nomor 67 Tahun 2016 Tentang Penanggulangan Tuberkulosis. Jakarta: Kementerian Kesehatan Republik Indonesia; 2016
6. Appleton S, Connell D, Singanayagam A, Bradley P, Pan D, Sanderson F et al. Evaluation of prediagnosis emergency department presentations in patients with active tuberculosis: the role of chest radiography, risk factors and symptoms. 2021
7. Atashi S, Izadi B, Jalilian S, Madani S, Farahani A, Mohajeri P. Evaluation of GeneXpert MTB/RIF for determination of rifampicin resistance among new tuberculosis cases in west and northwest Iran. New Microbes and New Infections. 2017;19:117-120
8. Bark C, Dietze R, Okwera A, Quelapio M, Thiel B, Johnson J. Clinical symptoms and microbiological outcomes in tuberculosis treatment trials. Tuberculosis. 2011;91(6):601-604
9. Barzegari S, Afshari M, Movahednia M, Moosazadeh M. Prevalence of anemia among patients with tuberculosis: A systematic review and meta-analysis. Indian Journal of Tuberculosis. 2019;66(2):299-307
10. Bhalla A, Goyal A, Guleria R, Gupta A. Chest tuberculosis: Radiological review and imaging recommendations. Indian Journal of Radiology and Imaging. 2015;25(3):213
11. Bowman A, Jain A, Baker B, Milano P, Terp S, Desai S. Chest X-Ray Findings in Emergency Department Patients Evaluated for Pulmonary Tuberculosis: The Experience of a Large Urban Academic Emergency Department. *Annals of Emergency Medicine*. 2015;66(4):S104
12. Cannas A, Calvo L, Chiacchio T, Cuzzi G, Vanini V, Lauria F et al. IP-10 detection in urine is associated with lung diseases. BMC Infectious Diseases. 2010;10(1)
13. Casha A, Scarci M. The link between tuberculosis and body mass index. *Journal of Thoracic Disease*. 2017;9(3):E301-E303
14. Chemeda A, Abebe T, Ameni G, Worku A, Mihret A. Utility of urine as a clinical specimen for the diagnosis of pulmonary tuberculosis in people living with HIV in Addis Ababa, Ethiopia. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*. 2019;17:100125
15. Ejeh E, Undiandeye A, Akinseye V, Okon K, Kazeem H, Kudi C et al. Diagnostic performance of GeneXpert and Ziehl-Neelson microscopy in the detection of tuberculosis in Benue State, Nigeria. *Alexandria Journal of Medicine*. 2018;54(4):529-533
16. Gopinath K, Singh S. Urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis. *International Journal of Infectious Diseases*. 2009;13(3):374-379
17. Habte D, Melese M, Hiruy N, Gashu Z, Jerene D, Moges F et al. The additional yield of GeneXpert MTB/RIF test in the diagnosis of pulmonary tuberculosis among household contacts of smear positive TB cases. *International Journal of Infectious Diseases*. 2016;49:179-184
18. Hong J, Jung G, Kim H, Kim Y, Lee H, Cho S et al. Efficacy of inducible protein 10 as a biomarker for the diagnosis of tuberculosis. *International Journal of Infectious Diseases*. 2012;16(12):e855-e859
19. Kim S, Kim J, Kim D, Kang Y, Bong S, Lee J et al. Urine IP-10 as a biomarker of therapeutic response in patients with active pulmonary tuberculosis. *BMC Infectious Diseases.* 2018;18(1)
20. Kim S, Ye S, Ha E, Chun E. Association of body mass index with incident tuberculosis in Korea. *PLOS One*. 2018;13(4):e0195104
21. Nonghanphithak D, Reechaipichitkul W, Namwat W, Naranbhai V, Faksri K. Chemokines additional to IFN-γ can be used to differentiate among Mycobacterium tuberculosis infection possibilities and provide evidence of an early clearance phenotype. *Tuberculosis*. 2017;105:28-34
22. Petrone L, Bondet V, Vanini V, Cuzzi G, Palmieri F, Palucci I et al. First description of agonist and antagonist IP-10 in urine of patients with active TB. *International Journal of Infectious Diseases*. 2019;78:15-21
23. Rohini K, Surekha Bhat M, Srikumar P, Mahesh Kumar A. Assessment of Hematological Parameters in Pulmonary Tuberculosis Patients. Indian Journal of Clinical Biochemistry. 2015;31(3):332-335

24. Rohini K, Surekha Bhat M, Srikumar P, Mahesh Kumar A. Assessment of Hematological Parameters in Pulmonary Tuberculosis Patients. *Indian Journal of Clinical Biochemistry*. 2015;31(3):332-335
25. Chandni R., Rajan G., Udayabhaskaran V. Extra pulmonary tuberculosis presenting as fever with massive splenomegaly and pancytopenia. *IDCases*. 2016; 4: 20-22
26. Shao Y, Peng H, Chen C, Zhu T, Ji M, Jiang W et al. Evaluation of GeneXpert MTB/RIF for detection of pulmonary tuberculosis at peripheral tuberculosis clinics. *Microbial Pathogenesis*. 2017;105:260-263.
27. Singla R, Raghu B, Gupta A, Caminero J, Sethi P, Tayal D et al. Risk factors for early mortality in patients with pulmonary tuberculosis admitted to the emergency room. *Pulmonology*. 2021;27(1):35-42
28. Wikanningtyas T, Hatta M, Massi M, Pratiwi I, in pulmonary tuberculosis patients based on the microscopic sputum examination. *Enfermería Clínica*. 2020;30:243-246
29. Yen Y, Chuang P, Yen M, Lin S, Chuang P, Yuan M et al. Association of Body Mass Index With Tuberculosis Mortality. *Medicine*. 2016;95(1):e2300
30. Yeong C, Byrne A, Cho J, Sintchenko V, Crighton T, Marais B. Use of GeneXpert MTB/RIF on a single pooled sputum specimen to exclude pulmonary tuberculosis among hospital inpatients placed in respiratory isolation. *International Journal of Infectious Diseases*. 2020;92:175-180
31. Zhang H, Li X, Xin H, Li H, Li M, Lu W et al. Association of Body Mass Index with the Tuberculosis Infection: a Population-based Study among 17796 Adults in Rural China. *Scientific Reports*. 2017;7(1)