European Journal of Biomedical Research
www.ej-biomed.org

Potential Immunological Biomarker for Diagnosis and Prognosis of Tuberculosis

Yassameen A. Hussain, Khairallah A. S. Mohammed, and Naael H. Ali

ABSTRACT

Tuberculosis (TB) is one of the most common infectious diseases in the world, which has led to numerous deaths. Hence, developing an efficient diagnostic method is essential to monitor and control such deadly infectious diseases. In the current study, the serum levels of four inflammatory markers (CXCL10, CXCL9, suPAR, and MMP9) and the expression NF-κB gene were evaluated as potential immunological markers for diagnosis and prognosis of tuberculosis, using ELISA and qPCR technique respectively. Thirty new TB patients and equal numbers of under treatment TB patients and control (healthy people) were conscripted in this study. The results showed significant differences in the serum level of CXCL10 among the three groups (p value 0.003) and between new and under treatment patients (P value 0.004). A significant difference in the CXCL9 level in the serum was observed between the new TB patients and the healthy group with p value 0.028 but didn't reach the significant level between the new and under treatment patients. The serum level of suPAR was higher in new patients (106.59pg/ml) followed by treated patients (89.66pg/ml) and lowest in healthy group (80.71pg/ml) but didn't reach the significant level. Also, the serum level of MMP-9 did not show a significant difference between the tested groups, but it was slightly higher in new patients (21.45ng/ml) compared to the healthy group (20.70ng/ml). The amount of NF-κB gene expression was significantly higher in new patients (8.21-fold change) than in under treatment patients (2.95-fold change) in comparing with healthy people.

Keywords: CXCL10, CXCL9, ELISA, tuberculosis, NF-κB gene.

Published Online: February 8, 2023

ISSN: 2796-0056

DOI: 10.24018/ejbiomed.2023.2.1.42

Y. A. Hussain*
Department of Medical Lab Technology, College of Health and Medical Technology, Southern Technical University, Iraq
(e-mail: yassmenalsayme@gmail.com)
K. A. S. Mohammed
Department of Medical Lab Technology, College of Health and Medical Technology, Southern Technical University, Iraq
(e-mail: dr.kmohammed@stu.edu.iq)
N. H. Ali
College of Medicine, University of Basra, Iraq
(e-mail: naael.ali@uobasrah.edu.iq)

****Corresponding Author***

I. Introduction

Tuberculosis is an infectious disease that is one of the leading causes of death and the leading causes of ill health worldwide. Most people (approximately 90%) who broaden the infection are adults, with greater instances amongst male than female. About 1/4 of the world's populace is inflamed with *M. tuberculosis* [1].

The most obvious effect is a sharp global decline in the number of persons newly diagnosed with and reported to have TB. This figure dropped from 7.1 million persons in 2019 to 5.8 million in 2020, an 18% drop from the 2012 level and well below the approximately 10 million persons who developed Tuberculosis in 2020. Sixteen nations accounted for 93% of this reduction, with India, Indonesia, and the Philippines the hardest hit.

Approximately (5–10%) of the people infected with bacteria develop the sickness throughout the primary 2–5 years once infection. In other infected people, the innate immune response will either absolutely dispose of the infection without leaving a touch of immunological response (resistance to TB infection) or reason of continual immune response to M. tuberculosis antigens without clinical evidence of active disease [2], [3]. The World Health Organization (WHO) strategy to end TB has set targets to reduce TB incidence would be reduced by 90% and TB deaths by 95% globally in 'by 2035. If there is any chance of achieving these ambitious goals, new tools will become available to fight this devastating disease. These include an urgent need for better diagnostic tests, shorter treatment regimens and more effective vaccines [4].

In Iraq, tuberculosis is still a significant public health issue. The country, which accounts for 3% of all cases, is one of seven in the Eastern Mediterranean area with a significant burden of TB. In Iraq, there are thought to be 20,000 TB cases. Between 200 and 4000 people are thought to die each year [5], [6]. Governing Tuberculosis infection, facing stagnant challenges associate rising issue of MDR (drug-resistant strains), evolution of recent treatments, a pressing want for intervention of LTBI (latent Tuberculosis infection), an absence of distinguished biomarkers for prognosis and diagnosis, etc. [7], [8].

The immune system is amongst the maximum vital inner danger elements. Adaptive cell-mediated immunity and Innate immunity are powerful instruments in protective the host in opposition to evolution of Tuberculosis by the role of CD4+T lymphocytes and Macrophages, along with formation of granuloma. They are the spine of immune protection in opposition to MTB and their position in controlling Tuberculosis is properly defined the characteristic of those cells in opposition to inhaled pathogens are managed through local surroundings of cytokines, which exert immune inflammatory (stimulator) and anti-inflammatory (inhibitory or regulatory) activities [19].

Throughout Tuberculosis infection, chemokines play an important role in mediating innate immunity, cell proliferation and migration, inflammation, angiogenesis, etc. [10], [11]. In particular, innate cytokine/chemokine

pathways play essential roles in dominant primary infection and in promoting adaptation immune responses, Anyway, anti- the balanced activation between pro- and inflammatory cytokines/chemokines is necessary for mounting effective host resistance against Mycobacterium tuberculosis infection [12]. So that the immune system is amongst the maximum vital inner danger elements. Previous studies have measured and recognized numerous host biomarkers in entire blood, serum, plasma, saliva, and cerebrospinal fluid lifestyle supernatants. Some of those proteins or biosignatures have proven top potentialities within side the analysis of diverse sorts of tuberculosis [13]–[16]. The current study is grounded on the immunological biomarkers and their role in diagnosing or prognosis tuberculosis, based on previous studies that demonstrated the importance of these immune indicators in diagnosing tuberculosis.

The aim of this study was to identify potential serum based immunological biomarkers for diagnosis and monitoring response to ant-tuberculosis treatment and to study the correlation between NF-κB gene expression and the levels of these biomarkers in TB patients.

II. Materials And Methods

Samples of blood were collected from pulmonary tuberculosis patients in the Advisory Clinic for Chest Disease and Respiratory (ACCDR), the only health center that deals with TB patients in Basra province, from January 2022 to May 2022. Thirty new diagnosed patients' equal numbers of treated patients and apparently healthy people were recruited in this study and consent form was obtained from each participant. Tuberculosis patients were diagnosed through direct examination of sputum Acid Fast Bacillus (AFB) and molecular examination using Gen-x-pert. Follow-up patients were selected from TB patients who were already diagnosed and had taken their treatment for more than three months.

A. Assessment of Immunological Markers in Serum

The levels of four inflammatory markers in serum were evaluated by using ELISA (Enzyme–linked Immunosorbent assay) with specific commercial kits as following: CXCL10, CXCL9, MMP-9 and suPAR (Sunlong Biotech, China) all the ELISA procedures were carried out according to manufacture instructions.

B. RNA Extraction

The ribonucleic acid (RNA) was extracted from blood by using Kit GENE ZO "Tri RNA pure Kit" (Geneaid, Taiwan) according to the manufacture's guideline. A nanodrop spectrophotometer was used to determine the concentration and the purity of extracted RNA. The extracted RNA was used as template and subjected to reverse transcription process by the first conversion of RNA was used template in to synthesize complementary DNA (cDNA) by transcription enzyme.

The analysis of gene expression of NF-κB was performed by using a real time PCR machine (Applied Biosystem, USA). The reaction tube contained (10µl of GoTaq® qPCR Master Mix with SYBR® Green (Promega, USA), sample cDNA (2µl), forward primer (1µl), revers primer (1µl) and Nuclease-free-water (6µl)) a total is (20µL). Two sets of specific primers were used to amplify the NF-κB and reference gene (GAP-DH). The primers synthesized by OriGene Company (USA) Table I. The program of thermocycle machine was 95°C for 1 minute; 40 cycles of 95°C for 15 seconds, 55-63°C for 1 minute, 95°C for 15 seconds. The quantity of NF-κB expression in the blood was determined as units relative to the quantity of GAP-DH expression which was calculated using ΔΔCT formula [17].

TABLE I: PCR Primers for Amplification the Gene Encode NF-Kb

Gene	Primer	Sequence (5' to 3')	Reference
NF-kB	Forward	GCAGCACTACTTCTTGACCACC	Ref: (Zhao & Erle,2018) [18]
	Reverse	TCTGCTCCTGAGCATTGACGTC	
GAP-DH	Forward	TTCCAATATGATTCCACCCA	
	Reverse	GATCTCGCTCCTGGAAGATG	

C. Statistical Analysis

For the purpose of statistical analysis, the Statistical Package for the Social Sciences (SPSS), versions 24 were used. To describe quantitative data, the mean±standard deviation, median and minimum- maximum values were calculated. For the description of qualitative data absolute numbers with percentage were used. Normality of distribution was tested using appropriate tests including Kruskal Wallis and Mann Whitney tests. The probability value of < 0.05 was considered significant.

III. Results

A. Comparison of Inflammatory Markers Levels between New TB Patients, under Treatment TB Patients and Control (Healthy People)

In the present study, CXCL10 serum level showed a significant change between the three groups (control, new and treated TB patients (p value=0.0003)) with highest level in new patients as shown in Table II.

TABLE II: Median Levels and Range of CXCL10 In Serum for TB Patients and Healthy Group.

Inflammatory makers (pg/ m)	Groups			P. value
	New TB Patients	Under treatment TB Patients	Healthy people	
CXCL10	22.09 (7.53–28.42)	15.14 (11.53–30.12)	19.07 (0.96–25.68)	0.003*
New TB patients VS Under treatment patients				0.004**
New TB patients VS healthy people				0.003**

*Kruskal Wallis Test, **Mann-Whitney U Test Sig.

Additionally, CXCL10 serum level in new patients was significantly higher than that under treatment TB patients and healthy people with p value 0.004 and 0.003 respectively. As shown in Table II.

Regarding serum level of CXCL9, MMP-9 and suPAR, the results showed a noticeable increase in serum level of these markers in new TB patients comparing to under treatment patients and healthy people (Table III). CXCL9 serum level showed no significant difference between the three groups (healthy people, new and treated TB patients) with p value=0.084. Also, further comparing the serum level

value of this marker between new and healthy people was found a significant change with p value=0.028. While there is no significant difference in both MMP-9 and suPAR in p values between three groups and any two groups (Table III).

TABLE III: MEDIAN LEVELS AND RANGE OF CXCL9, MMP-9 AND SUPAR IN SERUM FOR TB PATIENTS AND HEALTHY GROUP

Inflammatory makers	Groups			P. value
	New TB Patients	Under Treatment TB Patients	Healthy people	
CXCL9 (ng/ml)	3.17 (1.72–53.42)	3.12 (1.40–6.51)	2.89 (0.68–7.00)	0.084*
New TB patients VS Under Treatment TB patients				0.322**
New TB patients VS Healthy people				0.028**
MMP-9 (ng/ml)	21.45 (8.75–139.61)	20.32 (10.00–39.60)	20.70 (0.88–53.95)	0.835*
New TB patients VS Under Treatment TB patients				0.848**
New TB patients VS Healthy people				0.559**
suPAR (pg/ml)	106.59 (39.59–1345.11)	89.66 (26.64–167.51)	80.71 (6.09–226.14)	0.184*
New TB patients VS Under Treatment TB patients				0.280**
New TB patients VS Healthy people				0.081**

B. Comparison the Expression of NF-Kb in TB Patients According to the Healthy People

The present study in Tuberculosis patients indicates that the amounts of NF-κB expression in new TB patients was significantly higher than in the under-treatment patients (p value=0.0001). Likewise, the comparison was made between each two groups separately, where we found that there is a high significant difference in p values, where the p value was (0.0001) between the new group and the healthy group, while it was (0.002) between the under-treatment group and the healthy group (Table IV).

TABLE V: NF- KB GENE EXPRESSION IN TB PATIENTS ACCORDING TO THE HEALTHY PEOPLE

Groups	NF-κB gene expression (fold)	P.value
New TB Patients	8.21 (0.05–122.70)	
Under Treatment TB patients	2.95 (0.009–50.52)	0.0001*
New TB Patients VS Healthy people		0.0001**
Under Treatment TB patients VS Healthy people		0.002**

*Kruskal Wallis Test, **Mann-Whitney U Test Sig.

IV. DISCUSSION

A. The Importance of Estimating the Level of Inflammatory Markers in Patients with Tuberculosis

According to specialist consultation in evaluating patients' responses to anti-TB treatment and verifying the sterilizing efficacy of the medications, the development of tuberculosis (TB) biomarkers may have an impact on standard clinical practice and possibly help to identify novel clinical endpoints [19,20,21]. If new biomarkers are discovered, they may aid in TB treatment prognosis and therapy modification decisions [22], [23]. Recent research has found that a number of chemokines have a role in luring different immune cells to the lung through tuberculosis infection [24]. Additional chemokines including CXCL10 and CXCL9 have been suggested to have a part in TB susceptibility [25].

What we found in our current study, regarding CXCL10, was a significant increase in the p value among the three groups (p value=0.003) and also when comparing the new patients with the healthy people (p value=0.003) and the new patients with under treatment patients (p value=0.004), and this explains the importance of this marker in diagnosing tuberculosis disease, as well as its effectiveness in follow up the progress of patients in treatment, which decreases after taking the appropriate treatment for a period of time.

our findings of this study agreed with [26], CXCL10 has been the focus of a meta-analysis that included data from 18 studies with a total of 2,836 people, demonstrating that IP-10 may be used as a diagnostic marker to distinguish pulmonary tuberculosis (PTB) from non-TB. Another comprehensive study and meta-analysis demonstrate that CXCL10 may be useful in the diagnosis of latent TB [27]. According to this research, active pulmonary TB patients with (ATB) had much higher levels of IP-10 in their sera than healthy controls (HC) [28].

Studies have revealed that active TB patients' serum levels of CXCL10 significantly increase following stimulation [29] this was similar to our current study, where this marker showed a significant difference in both under treatment and new patients compared to the healthy people. Following anti-TB medication, IP10 has been identified as a biomarker for improvement [30].

CXCL9 is an excellent biomarker to identify PTB from LTB, and studies in the past have demonstrated that it greatly decreases after treatment [31], [32] and for disease severity can also CXCL9 serves as a good plasma biomarker [33].

In line with above previous studies, we found there is a significant difference (p value=0.028) in the level of this Marker (CXCL9) between new patients and healthy people in our findings, and this matches with the previous study [9]. However, it in the term of CXCL9 level in tuberculosis patients before and after treatment, there was a slight decreasing in the serum level of this biomarker but did not reach a significant level and the reason may be the small sample size of under treatment TB patients' number (n=30).

Previous research has demonstrated that infection with MTB leads to stimulation and causes MMPs production. As regulatory mechanisms drive MMP-9, it has been suggested that reducing MMP-9 activity is a viable aim as an additional therapy for limiting Tuberculosis immunopathology. MMP-9 has been demonstrated to play a important role in TB pathogenesis [34]. According to previous researches, early MMPs activity are a crucial element of resistance to pulmonary mycobacterial infection, and MMP9 is particularly important for attracting macrophages and tissue remodeling so that tight, well-organized granulomas can develop [35]. Our results showed that MMP-9 levels did not significantly change in new or under treatment patients. In a study on tuberculous meningitis (TBM) patients, Majeed S *et al*. [36] made a similar report, stating that levels of MMP-9 were unaffected by anti-tuberculosis medications, which explains why MTB

patients continued to have lifelong neurological defects even after the bacillary infection was cleared. Contrarily, several reports indicate that during the intensive phase of treatment for infiltrative pulmonary tuberculosis, serum MMP-9 levels were increased [37]. Conversely, in biopsy samples taken from PTB patients, MMP-9 gene expression in pulmonary epithelial cells infected with Mycobacterium tuberculosis was found to be decreased [38]. According to the results shown by our current study, there is no difference in the level of MMP-9 in the serum of patients compared to the healthy people, and also when comparing each two groups separately, no significant difference appeared in the p values. This is consistent with a previous study conducted on tuberculosis patients, where the MMP-9 level in the serum was measured before taking treatment for new patients and follow-up patients [39]. However, these results need to be confirmed with a larger sample size.

SuPAR, the soluble version of urokinase-type plasminogen activator receptor (uPAR). The membrane receptor (uPAR), which is expressed in cell types including neutrophils, endothelial cells, macrophages and monocytes binds to the urokinase-type plasminogen activator (uPA), which is released by PMN (polymorphonuclear neutrophils) and macrophages. suPAR potential has been observed as a general biomarker in the diagnosis, prognosis, and follow-up of lung disease therapy; Tuberculosis patients often have greater suPAR expression than healthy people [40].

Our results showed that there is an increase in serum level of suPAR in the new tuberculosis patients compared to the control group and under treatment patients. These results are completely consistent with the previous studies which showed high serum level of suPAR in new patients than in healthy and undertreatment patients but did not reach significant differences. This finding indicates the importance of this marker in diagnosing the disease and as an important tool in observing the progress of treatment in under treatment TB patients. SuPAR might be used as a test step but could not be cost effective particularly in low-income countries.

B. Comparison of NF-Kb Gene Expression in New TB Patients and under Treatment TB Patients Comparing to the Healthy People

Studies have shown that NF-κB is a double-edged sword, as it helps TNF-α secretion along with a series of cytokines and chemokines that all work on regulating the immune response to MTB infection and activating macrophages to kill engulfed bacteria, leading to the apoptosis of infected macrophage [41], [42] On the other hand, NF-κB activation helps improve the chance for bacteria to survive inside the macrophage and prevent the process of apoptosis [43], [44] as it improves tissue invasion [45] and this is similar to what reported in Loeuillet's research [46] who showed that the NF-κB prevents cells infected with bacteria from the process of apoptosis and this could provide a favorable environment for bacteria to multiply and thus avoid the body's immune response. Previous studies showed that patients with active pulmonary TB have elevated NF-κB activity in alveolar macrophages, which increases the production of pro-inflammatory cytokines and chemokines [47], [48].

In our current study, we noticed an increase in the NF-κB gene expression level in new patients (8.21-fold change) and a decrease after taking the appropriate treatment in the under treatment patients (2.95-fold change) in comparing with the healthy group (p value=0.0001) , new TB patient Vs healthy people (p value=0,0001), and under treatment patients Vs healthy group (p value 0.002) this is consistent with several previous studies on the NF-κB level in tuberculosis patients [49] where it was found that the level of NF-κB gene expression in active tuberculosis patients shows a significant difference compared to control and this is similar to our current results confirms that bacteria inhibit macrophages apoptosis during early infection by up-regulating the NF-κB signaling pathway also when comparing active tuberculosis patients with the latent TB patients in another study [44] there is down-regulating for NF-Kb in Latent TB patients and this means that the immune system inhibits NF-κB activation and this reduces chance of surviving MTB by improving both apoptosis and autophagy of the infected macrophage. Based on the results obtained in this study and previous research, It is likely that NF-κB can be employed as a possible biomarker and a crucial treatment target.

V. Conclusion

Evaluation of serum levels of CXCL10, CXCL9, suPAR and MMP-9) and NF-κB gene expression in TB patients showed the importance of these marker as possible indication in diagnosis and follow up TB patients. However, further study with larger sample sizes including multi-drug resistant TB patients is required to confirm and extend the results that obtained in this study.

Acknowledgment

All the authors extend their sincere thanks to the entire staff of the Advisory Clinic for Chest Diseases and Respiratory, Ms. Zahra Header for her assistance in the molecular analysis and to Prof. Dr. Alaa Hussain Abid for his assistance in the statistical analysis of research data.

Funding

The funding was performed by the author.

Conflict of Interest

The authors confirmed there is no conflict of interest.

References

[1] WHO. global tuberculosis report 2021. *Geneva: World Health Organization*, p. 57. 2021.

[2] Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, *et al*. Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. *Nat Rev Immuno*. 2018 Jun 12;18(9):575–89.

[3] Saito H, Timurkaynak F, Borzykowski T, Kilpatrick C, Pires D, Allegranzi B, *et al*. It's in your hands-prevent sepsis in health care. 5th May 2018 World Health Organization (WHO) SAVE LIVES: Clean Your Hands Campaign. *Kli Derg/Kli J*. 2018 Jun 6;2–3.

[4] Raviglione MC. End tb–the new who strategy in the sdg era and the contributions from the Russian federation. *Tube and lung dis*. 2016 Nov;94(11):7–15.
[5] Durib, A. K. Prevalence of Tuberculosis in Baghdad, Iraq 2012-2016. *Inter J of Sci and Res Pub*. 2018; 8: 565–570.
[6] Al-Dahmoshi, H. O., Al-Khafaji, N. S. K., & Al-Allak, M. H. Iraqi tuberculosis (2003–2017): a silent hindrance infection. *J Bac Mycol Open Access*. 2019; 7(4): 98–100.
[7] Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, *et al*. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lan Res Med* [Internet]. 2017 Apr 1;5(4):291–360. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2213260017300796.
[8] Barry CE, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, *et al*. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Micro*. 2009 Oct 26;7(12):845–55.
[9] Ali ZA, Mankhi AA, Ad'hiah AH. Significance of the chemokine CXCL10 and human beta-defensin-3 as biomarkers of pulmonary tuberculosis. *Tube*. 2021 May; 128:102078.
[10] Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and chemokines in mycobacterium tuberculosis infection. *Micro spec* [Internet]. 2016;4(5): 10. 1128/microbiolspec.TBTB2-00182016. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27763255.
[11] Torraca V, Tulotta C, Snaar-Jagalska BE, Meijer AH. The chemokine receptor CXCR4 promotes granuloma formation by sustaining a mycobacteria-induced angiogenesis programme. *Sci Rep*. 2017 Mar 23;7(1).
[12] Cooper AM, Mayer-Barber KD, Sher A. Role of innate cytokines in mycobacterial infection. *Muc Immun*. 2011 Mar 23;4(3):252–60.
[13] Manyelo CM, Solomons RS, Snyders CI, Manngo PM, Mutavhatsindi H, Kriel B, *et al*. Application of cerebrospinal fluid host protein biosignatures in the diagnosis of tuberculous meningitis in children from a high burden setting. *Med of Inflam*. 2019 Apr 16; 2019:1–11.
[14] MacLean E, Broger T, Yerlikaya S, Fernandez-Carballo BL, Pai M, Denkinger CM. A systematic review of biomarkers to detect active tuberculosis. *Nat Micro*. 2019 Feb 25;4(5):748–58.
[15] Chegou NN, Sutherland JS, Namuganga A-R, Corstjens PL, Geluk A, Gebremichael G, *et al*. Africa-wide evaluation of host biomarkers in QuantiFERON supernatants for the diagnosis of pulmonary tuberculosis. *Sci Rep*. 2018 Feb 8;8(1).
[16] Chegou NN, Black GF, Kidd M, van Helden PD, Walzl G. Host markers in Quantiferon supernatants differentiate active TB from latent TB infection: preliminary report. *BMC Pulm Med*. 2009 May 16;9(1).
[17] Livak, K. J., Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*. 2001; 25(4), 402–408.
[18] Zhao, W., Erle, D. J. Widespread effects of chemokine 3´ untranslated regions on miRNA degradation and protein production in human cells. *J of Immun*. 2018; 201(3), 1053-1061.
[19] Djoba Siawaya JF, Bapela NB, Ronacher K, Veenstra H, Kidd M, Gie R, *et al*. Immune parameters as markers of tuberculosis extent of disease and early prediction of anti-tuberculosis chemotherapy response. *J of Infec*. 2008 May;56(5):340–7.
[20] Walzl G, Ronacher K, Djoba Siawaya JF, Dockrell HM. Biomarkers for TB treatment response: Challenges and future strategies. *J of Infec*. 2008 Aug;57(2):103–9.
[21] Cilliers K, Menezes A, Webber T, Dockrell HM, Cliff JM, Kleynhans L, *et al*. Mycobacterium tuberculosis-stimulated whole blood culture to detect host biosignatures for tuberculosis treatment response. *Tube*. 2021 May; 128:102082.
[22] Musteikienė G, Miliauskas S, Zaveckienė J, Žemaitis M, Vitkauskienė A. Factors associated with sputum culture conversion in patients with pulmonary tuberculosis. *Med*. 2017;53(6):386–93.
[23] Musteikienė G, Miliauskas S, Zaveckienė J, Urbonienė D, Vitkauskienė A, Žemaitis M, *et al*. Is analysis of inflammatory biomarkers and lymphocyte subpopulations useful in prediction of tuberculosis treatment outcomes. *J of Clin Tube and Other Mycobac Dis*. 2021 Dec; 25:100275.
[24] Monin L, Khader SA. Chemokines in tuberculosis: The good, the bad and the ugly. *Sem in immun* [Internet]. 2014 Dec 1 [cited 2021 May 27];26(6):552–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4314384/.
[25] Domingo-Gonzalez, R., Prince, O., Cooper, A., Khader, S. A. *Cytokines and chemokines in Mycobacterium tuberculosis infection*. Tube and the tub bac. pp. 33–72. 2019.
[26] Qiu X, Xiong T, Su X, Qu Y, Ge L, Yue Y, *et al*. Accumulate evidence for IP-10 in diagnosing pulmonary tuberculosis. *BMC Infec Dis*. 2019 Oct 30;19(1).
[27] Qiu X, Tang Y, Zou R, Zeng Y, Yue Y, Li W, *et al*. Diagnostic accuracy of interferon-gamma-induced protein 10 for differentiating active tuberculosis from latent tuberculosis: A meta-analysis. *Sci Rep*. 2019 Aug 6;9(1).
[28] Wawrocki S, Seweryn M, Kielnierowski G, Rudnicka W, Wlodarczyk M, Druszczynska M. IL-18/IL-37/IP-10 signalling complex as a potential biomarker for discriminating active and latent TB. Subbian S, editor. *PLOS ONE*. 2019 Dec 10;14(12): e0225556.
[29] Tebruegge M, Dutta B, Donath S, Ritz N, Forbes B, Camacho-Badilla K, *et al*. Mycobacteria-Specific Cytokine Responses Detect Tuberculosis Infection and Distinguish Latent from Active Tuberculous. *Amer J of Res and Crit Care Med*. 2015 Aug 15;192(4):485–99.
[30] Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S. Plasma Proinflammatory Cytokines Are Markers of Disease Severity and Bacterial Burden in Pulmonary Tuberculosis. *Open Forum Infec Dis*. 2019 May 31;6(7).
[31] Wergeland I, Pullar N, Assmus J, Ueland T, Tonby K, Feruglio S, *et al*. IP-10 differentiates between active and latent tuberculosis irrespective of HIV status and declines during therapy. *J of Infec*. 2015 Apr;70(4):381–91.
[32] Zhao Y, Yang X, Zhang X, Yu Q, Zhao P, Wang J, et al. IP-10 and RANTES as biomarkers for pulmonary tuberculosis diagnosis and monitoring. *Tube*. 2018 Jul; 111:45–53.
[33] Kumar NP, Moideen K, Nancy A, Viswanathan V, Shruthi BS, Sivakumar S, et al. Plasma chemokines are biomarkers of disease severity, higher bacterial burden, and delayed sputum culture conversion in pulmonary tuberculosis. *Sci Rep*. 2019 Dec;9(1).
[34] Elkington PT, Ugarte-Gil CA, Friedland JS. Matrix metalloproteinases in tuberculosis. *Eur Res J*. 2011 Jun 9;38(2):456–64.
[35] Taylor JL, Hattle JM, Dreitz SA, Troudt JM, Izzo LS, Basaraba RJ, et al. Role for Matrix Metalloproteinase 9 in Granuloma Formation during Pulmonary Mycobacterium tuberculosis Infection. *Infec and Immu* [Internet]. 2006 Sep 18;74(11):6135–44. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1695484/.
[36] Majeed S, Singh P, Sharma N, Sharma S. Title: role of matrix metalloproteinase−9 in progression of tuberculous meningitis: a pilot study in patients at different stages of the disease. *BMC Infec Dis*. 2016 Nov 29;16(1).
[37] Esmedlyaeva DS, Alexeyeva NP, Sapozhnikova NV, Dyakova ME, Perova TL, Kiryukhina LD, et al. The system of matrix metalloproteinases and their role in patients with pulmonary tuberculosis. *Biomed Khim*. 2016;62(5):593–8.
[38] Singh, Shivani, *et al*. Interleukin-17 regulates matrix metalloproteinase activity in human pulmonary tuberculosis. *The J of patho*.2018; 244(3): 311–322.
[39] Warrier AG, Kumarchandra R, Sudha K, Jayashree BB, Durgarao Y. Role of MMP 9 in the diagnosis and management of pulmonary tuberculosis and its association with nutritional status. *Biomed*. 2021 Apr 2;41(1):36–41.
[40] Soluble urokinase-type plasminogen activator receptor (suPAR) in multiple respiratory diseases. *Rec & Clin Inves*. 2015 Jan 29.
[41] Harris J, Hope Jayne C., Keane J. Tumor Necrosis Factor Blockers Influence Macrophage Responses to Mycobacterium tuberculosis. *The J of Infec Dis*. 2008 Dec 15;198(12):1842–50.
[42] Fallahi-Sichani M, Kirschner DE, Linderman JJ. NF-κB signaling dynamics play a key role in infection control in tuberculosis. *Fron in Physio*. 2012;3.
[43] Tato CM, Hunter CA. Host-Pathogen Interactions: Subversion and Utilization of the NF-B Pathway during Infection. *Infec and Immun*. 2002 Jul 1;70(7):3311–7.
[44] Bai X, Feldman NE, Chmura K, Ovrutsky AR, Su W-L, Griffin L, *et al*. Inhibition of Nuclear Factor-Kappa B Activation Decreases Survival of Mycobacterium tuberculosis in Human Macrophages. Bozza PT, editor. *PLoS ONE* [Internet]. 2013 Apr 25 [cited 2019 Apr 9];8(4): e61925. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3636238/.
[45] Philpott DJ, Yamaoka S, Israël A, Sansonetti PJ. InvasiveShigella flexneriActivates NF-κB through a lipopolysaccharide-dependent innate intracellular response and leads to il-8 expression in epithelial cells. *The J of Immuno*. 2000 Jul 15;165(2):903–14.
[46] Loeuillet C, Martinon F, Perez C, Munoz M, Thome M, Meylan PR. *Mycobacterium tuberculosis* subverts innate immunity to evade specific effectors. *The J of Immuno*. 2006 Oct 18;177(9):6245–55.

[47] Wayne LG, Sohaskey CD. Nonreplicating Persistence of Mycobacterium Tuberculosis. *Ann Rev of Micro*. 2001 Oct ;55(1):139–63.
[48] Wang C-H, Chou P-C, Chung F-T, Lin H-C, Huang K-H, Kuo H-P. Heat shock protein70 is implicated in modulating NF-κB activation in alveolar macrophages of patients with active pulmonary tuberculosis. *Sci Rep*. 2017 Apr 27;7(1).
[49] Alam A, Imam N, Siddiqui MF, Ali MdK, Ahmed MM, Ishrat R. Human gene expression profiling identifies key therapeutic targets in tuberculosis infection: A systematic network meta-analysis. *Infec, Gen and Evolu*. 2021 Jan; 87:104649.