تأثير تقنية متقدمة لوصف المستقبلات الشبيهة بالرسائل (TLR2وTLR4) في مرض السل

كاظم عباس مهدي*

احسان عيدان السيمري*

الملخص

تم إجراء دراسة حالة ضابطة بين مرضى السل الذين حضروا إلى المركز الاستشاري لأمراض الصدر والجهاز التنفسي في محافظة البصرة خلال الفترة من 1 سبتمبر 2020 إلى 1 يونيو 2021. من إجمالي عدد (176) مريض بالسل (TB) تم أخذها من محافظة البصرة التي شملتها الدراسة الحالية. في نتائج قياس التدفق الخلوي ، وجدت الدراسة الحالية أن TLR2 موجود على سطح الخلايا الوحيدة بنسبة 32.08٪ وفي المجموعة الضابطة 14.8٪ ، و TLR4ظهر في 3.4٪ بين مرضى السل وفي المجموعة الضابطة كان 0.8٪.

الكلمات المفتاحية : السل ، البصرة ، قياس التدفق الخلوي ، المستقبلات المشابهة

* قسم الأحياء الدقيقة ، جامعة البصرة ، كلية الطب ، البصرة ، العراق.

Impact of an advanced technique to characterize Toll- Like Receptors (TLR2 and TLR4) in tuberculosis

Ihsan Edan Alsaimary*
Kadhim Abbas Mahdi*

Abstract

A controlled case study has been carried among pateints with Tuberculosis ,who attended the Consultation Center of Chest and Respiratory diseases of Basrah province during 1st September 2020 to 1st June 2021. From total number of (176) patients with Tuberculosis (TB) were selected from Basrah province that are included in the present study. In flow cytometry results, the present study found that TLR2 was present on monocytes surface in percentage 32.08% and in control group was 14.8% , and TLR4 appeared in 3.4% among TB patients and in control group was 0.8%.

Key words: **tuberculosis , Basrah, flow cytometry , Toll- Like Receptors**

* Department of Microbiology, college of medicine, university of Basrah, Basrah, Iraq.

Introduction

Tuberculosis bacteria are disseminated by little droplets discharged into the air by coughing and sneezing, singing or simply talk from one person to the next, and from one person to the next. The neighboring people can respire and become infected with these bacteria [1]. An infectious disease, known as Tuberculosis (TB), remains one of the world's largest bacterial infections [2]. TB-related problems have been identified a recognized in the past, but their severity has been more recently emphasized because of emerging antibiotic resistance in TB and the risk of re-infection [3]. Innate immunity shown a major role in protecting the host from early infection with TB, as indicated by the majority of TB-exposed individuals being able naturally control the infection although a conspicuous delay of acquired immunity [1]. The immune system, including adaptive and innate immunological mechanisms, modulate host response to tuberculosis infection (both active and latent) [2]. To stop the successful incorporation of TB infection in the lungs, host immune cells, and various nonclassical immune cells in the airway are fortified with a clusters of cell-surface and intracellular Pattern Recognition Receptors (PRRs) to recognize the occupying of mycobacteria, such as Toll-like receptors. At the meeting of host mucosal immunity and TB pathogenes, these innate immune sensors play a vital role [3,4,5]. The early warning part of the recognizing bacteria was the natural immune system through its own receptors such as Toll-like receptors (TLRs), these were a gruop of distinct single membrane-spanning receptors consist of (1 to 10) types have been founded in humans, in both immune and non-immune cells and the (11 to 13) types in non-humans [6,7,8]. The Toll-like receptors that expressed on cell surface were TLRs (1, 2, 4, 5, and 6), while TLRs (3, 7, 8, and 9) founded absolutely inside endosomes and these who known to be involved in recognition of *Mycobacterium tuberculosis* (*MTB*) were TLR2, TLR4, TLR9 and probably TLR8 [2,4]. Normally TLRs play an important role in both innate immune responses and the induction of adaptive immunity to TB. Really, polymorphisms of TLRs have been related with mutated susceptibility to tuberculosis among different populations [9,10]. The TLRs are transmembrane proteins that illustrated as a key in the innate immune system considered pattern recognition receptors (PRRs), binding to Pathogen-Associated Molecular Patterns (PAMPs). Their function is Recognition of pathogens; and stimulation of immune responses directed against those pathogens [11,12]. The primary innate immune cells participating in TB infection are macrophages, neutrophils, dendritic cells, and natural killer cells. PRRs expressed on innate immune cells recognize PAMPs present in *MTB* and have an important function in the initiation responses of innate immunity [13]. *Mycobacterium tuberculosis* can escape immune responses [14]. And interrupt the crosstalk between acquired and innate immunities [15]. Host defense systems initiate various strategies for eliminating TB such as activating proinflammatory responses [17]. Producing reactive intermediates such as Reactive Oxygen Species (ROS) and reactive nitrogen species [18]. And inducing cell death to inhibit the spread of TB infection [19].

- Tuberculosis begins with ingestion of *Mycobacterium tuberculosis* through inhaled into the pulmonary alveoli. TB is identified by phagocytic cells of the innate immune system such as macrophages and denderitic cells (DCs), natural killer cells, and neutrophils, interact with various mycobacterial components, which represents the first line of host defense [22]. These cells express many Pattern Recognition receptors (PRRs), including Toll-like receptors (TLRs), C-lectin type receptors (CLRs), complement receptor 1 (CR1), complement receptoer 3 (CR3), dendritic cellspecific intracellular adhesion molecule-3–grabbing nonintegrin, mannose receptors, surfactant protein A receptors, class A scavenger receptors, mannose-binding lectin and NOD like receptors (NLRs), which are recognize antigenic molecules expressed by *Mycobacterium tuberculosis* called pathogen associated with the molecular pattern (PAMPs) [23,24,25,26].

The Toll-like receptors (TLRs) have an vital role in Mycobacterium infection, these receptors are associated with particular ligands exist on the bacteria to facilitate the absorption of *MTB* in to the cells, which leads to the inducement phagocytic cells to produce cytokines, chemokines which serve as a sign of infection and crucial to stimulate the adaptive immune defenses and to stop growing of bacteria. As a result, TLRs serve as a connection between innate and adaptive immune defenses against Mycobacterium infection [27,28,29]. The alveolar macrophages and dendritic cells with engulfed bacilli migrate to the regional lymph node and prime T cells (both CD4+ and CD8+) against mycobacterial antigens [30]. The specific immune response produces primed T cells which migrate back to the focus of infection, guided by the chemokines produced by the infected cells [31]. The accumulation of macrophages, T cells, and other host cells (dendritic cells, fibroblasts and endothelial cells) leads to the formation of granuloma at the site of infection [32,33,34,35,36]. The formation of granulomas is barriers away from the other lung tissue tuberculosis and limits the body bacterial spread, as well as the interaction of macrophages and other immune cells and cytokines that these cells produced [37]. The

CD4+ T lymphocytes which produce IFN-γ detect and destroy infected macrophages presented with *MTB* antigens [38-40]. The infection progression is halted; however, some resistant bacilli capable of surviving under the stressful conditions generated by the host escape killing and enter a state of dormancy and persist by avoiding elimination by the immune system [41-46].

Materials and methods

Sampling and source

This case control study was carried out in the province of Basrah between 1st September 2020 and 1st June 2021. During the process of collecting data, the patients' names, age, gender, marital status, medical family history, personal information and clinical disease findings were reported on a single questionnaire for each patient. Samples of blood have been gathered from the symptomatic patients of the Chest and Respiratory Diseases consultation center of the province of Basrah. Every samples of patients and control group were investigated in this study with age ranged from 14 years to equal or less than 78 years. Most of patients suffer symptoms like (Fever, chills, night sweats, loss of appetite, weight loss, fatigue). The blood samples were collected from patients after examination by the Pulmonologist and confirm as Tuberculosis according to clinical criteria.

Control Group

A total of 88 individuals without pulmonry problem, infectious diseases and allergies they were regarded as control group.

The number of patients group are calculated according to minimum size equation based on the ratio of disease which about 11 %.

Blood samples:

Five ml of venous blood was drawn by vein puncture using disposable syringes from each participant; 2 ml which will keep in EDTA tube and the other 3ml in disposable, non-pyrogenic, and non-endotoxin plastic tube which placed as a whole blood sample at room temperature for 2 hours and centrifugation for 20 minutes at approximately 1000 revolution per minute (rpm), blood collection tubes should be undergone centrifugation where the serum will obtained and preserved at (-20) ^{o}C till be used.

Flow cytometry instruments and kits :

Table (1) show the instrument and Kits of flow cytometry.

Table (1) Flow cytometry instrument and kits

Item	Model	Company	Country
Flow cytometry	Bricyte E6	Mindray	China
Anti-h TLR2	ABCW021805	Minneapolis	USA
Anti-h TLR4	ABOG0217081	Minneapolis	USA

The results

A case control study was carried on an overall cases of tuberculosis patients were (88) that taken from the Consultation Ceter of Chest and Respiratory diseases of Basrah province through period of 1st September 2020 to 1st June 2021, thier age were ranged (14 - 78) years. Cutch up with (88) individuals regarded as control group were checked and confirmed to be free from any respiratory diseases or any other health problems that also studied, the number of cases are obtained according to minimum size equation that depend on the ratio of disease.

Flow cytometry plots for TB patients and control group

Figure (1) shows the getting area of flow cytometry after lysis process of blood cells for control sample, flow cytometry divide the cells according to size and shape, the shape include the cytoplasmic contents of cell and their granules and also shape of nucleus therefore, because the sample was a blood samples after passing the lysing steps by breakdown of RBCs and Platelets and get only WBCs.

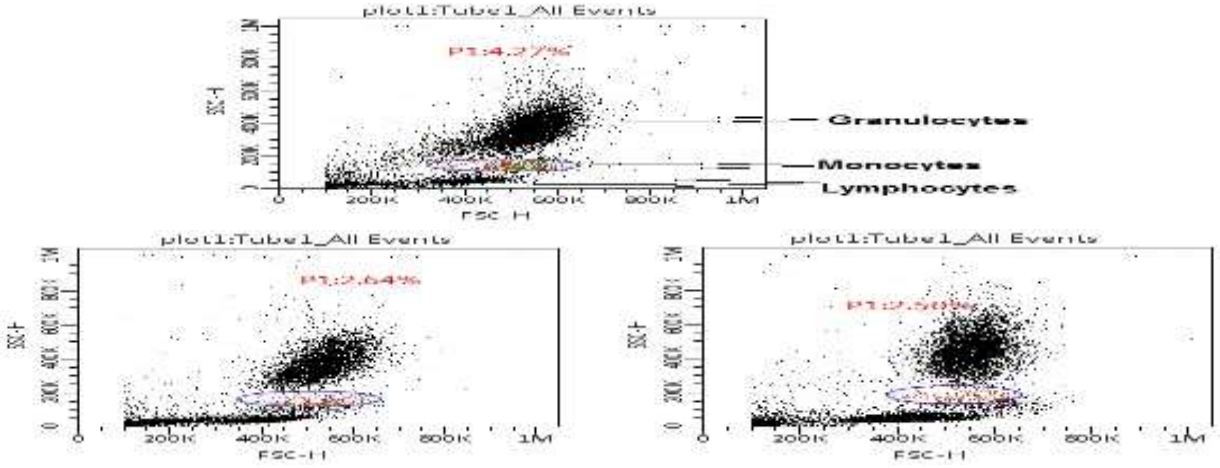
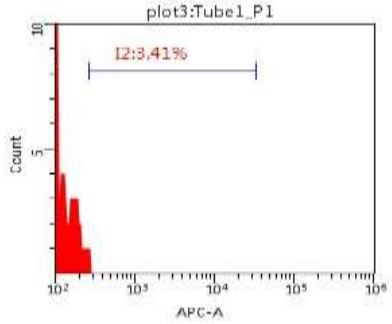


Figure (1) : Shown Getting area of flow cytometry after lysis process

Flow cytometry plots for TLR2 in TB patients and control group

Figure (2) shows histogram of flow cytometry that explains the availability of TLR2-CD marker for various samples with percentage of TLR2 for patients with tuberculosis and control group.



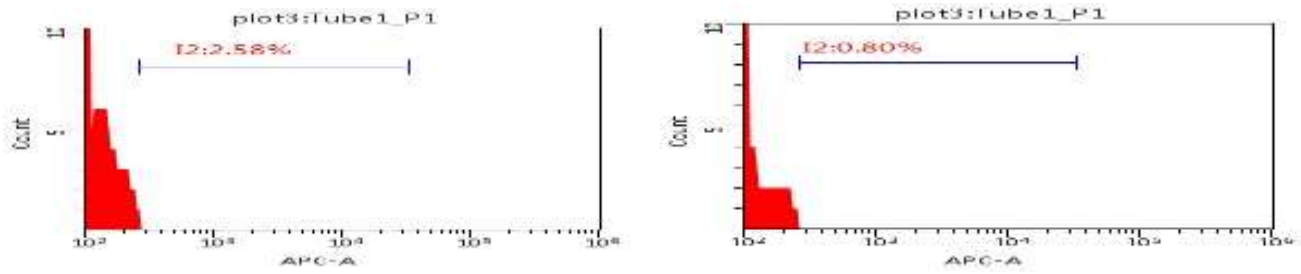


Figure (2) : Shown the availability of cells for certain CD marker for various samples with percentage of TLR2 for tuberculosis patients and control group.

Flow cytometry plots for TLR4 in TB patients and control group

Figure (3) shows histogram of flow cytometry that explains the availability of TLR4-CD marker for various samples with percentage of TLR4 for patients with tuberculosis and control group.

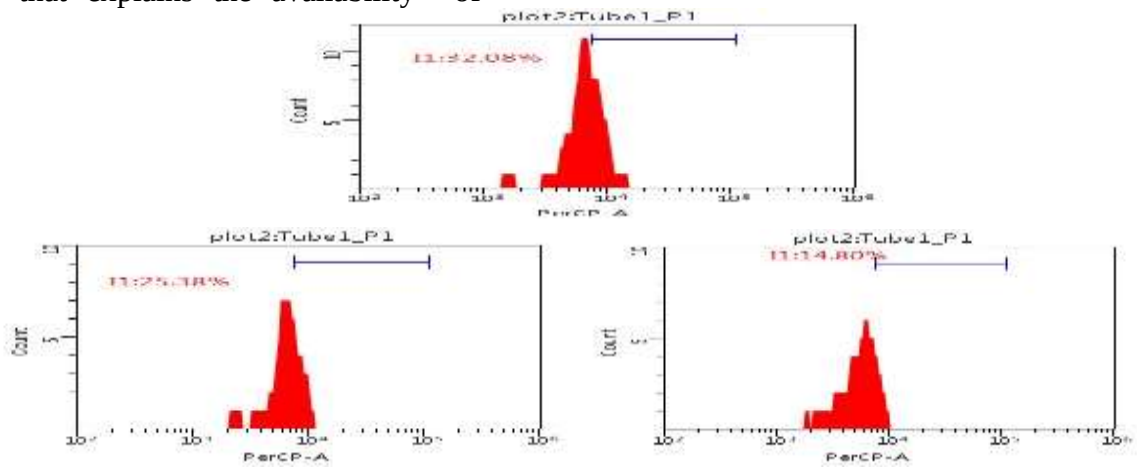


Figure (3) : Show the availability of cells for certain CD marker for various samples with percentage of TLR4 for tuberculosis patients and controls.

Number of TLR2 and TLR4 on Monocytes

Table (1) show the number and percentage of TLR2 and TLR4 among patients with tuberculosis and control group that found TLR2 in tuberculosis patients was 3.41% and in control group was 0.8%, and found TLR4 was 32.08% with tuberculosis patients and in control group was 14.8% by total monocytes from total white blood cells.

Table(1): show the number and percentage of TLR2 and TLR4 on Monocytes in blood component of tuberculosis patients in comparison with control.

TLRs on Monocytes		TB Patient	Control
TLR2	%	32.08	14.8
	No.of monocytes from total monocytes× 10^9/ L	0.133	0.048
TLR4	%	3.41	0.8
	No. of monocytes from total monocytes× 10^9/ L	0.014	0.002
Total Monocyte From total	%	3.9	6.1
	No. of monocytes × 10^9/ L	0.414	0.324

WBCs			
Total No. of WBCs × 10^9/ L		10.61	5.32

W.B.Cs counts among tuberculosis patients and control group:

Table (2) show number of W.B.Cs among patients with tuberculosis and control group. That found the number of W.B.Cs in tuberculosis patients was 10.61×10^9/L with Monocytes ratio 3.9% , Lymphocytes ratio 30.0% and Granulocytes ratio 66.1% .

Table (2): illustrate the number and percentage of white blood cells.

WBCs		Normal Values	TB Patient	Control
Total WBCs	count×10^9 / L	4.0 – 10.0	10.61	5.32
Monocyte	%	3.0 – 8.0	3.9	6.1
	count×10^9 / L	1.0 – 1.5	0.412	0.324
Lymphocytes	%	20.0 – 40.0	30.0	46.5
	count×10^9 / L	0.5 – 1.0	3.183	2.473
Granulocytes	%	50.0 – 70.0	66.1	47.4
	count×10^9 / L	1.2 – 8.0	7.013	2.521
Total	%	100.0 %	100.0 %	100.0 %

The Discussion

Tuberculosis is a contagious disease that is remains one of the major bacterial infections worldwide [47]. Tuberculosis-related problems were identified in the past but their severity was impressive as TB antibiotic resistance was emerging and reinfection risk [48]. A case control study was carried on an overall cases of tuberculosis patients were (88), thier age were arounded (14 - 78) years. In addition to (88) persons observed as control group, in this investigation the highest age group of patients with tuberculosis was (20-29) years were 23 (26.1 %) and fallowed by the fourth decades (30-39) were 16 (18.2 %) from total study patients, at the fifth decades (40-49) were 14 (15.9%), closely with it the second decades (10-19) were 13 (14.8%), while less cases of tuberculosis appeared at the age (>60) were 10 (11.4%) from total study cases. This results similler with [49] that observed during adolescence (age 15–19 years), there is a rapid increase in risk with a second peak between the ages of (20–30) years, this supported by the study of [50]that found TB primerly affect adolescent and adults, and other studies evidences our results and give more explianation about distribution of tuberculosis within age group like [51] that conclude the age distribution of tuberculosis case s mirrors global patterns, with a low number of cases in childhood and a high number in young adulthood. Other study disease of poverty affecting mostly young adults in their most productive years. The vast majority of TB deaths are in the developing world[52]. And other study [53] saying that the incidence of TB varies with age, while the study of [54] that found TB is mainly a disease of older people, or of the immune compromised.

TLR2 a member of pattern recognition receptors (PRRs) plays critical role in host immune response against TB infection. TLR2, which is a well-known receptor forming with TLR1 or TLR6, heterodimers, involves the recognition and response of innate immune cells the dendritic and macrophagous cells. TLR2 is the central receptor for mycobacterial detection in particular [59,60]. TLR2 is used to recognize the presence of fungi, parasites and virus in a broad range of bacteria [61]. In the current study documented that the mean concentrations of TLR-2 (ng/ml) among male and female of tuberculosis patients (0.63±0.26) (0.67±0.26) respectively, was higher than male and female of control group (0.22±0.10) (0.20±0.13) respectively, statistically the differences was highly significant. In these results that the concentration of TLR2 in TB female patients slightly more than male, suggested may according to thier hormonical activity differences. In other studies [62] observed that TLRs can prompt Tlymphocyte activation, adjust and ruler the aquired

immunity, and keep the body's immune system balanced. In other way [63] shown that TLR2 and TLR4 participate in recognizing and promoting inflammatory reactions to tuberculosis and associated metabolites. In study of [64] was carried out to TLR2, TLR4, TNF-α, IFN-α, IL-2, IL-6, and IL-10 expressions were investigated in HIV patients infected with TB. These findings suggested that concentration of TLR2 associated with the activity of TB infection and the patient immunity responses after clear comparison with TLR2 concentration of control group. In addition study TLR2 is thought to be important to initiate innate host protection through its stimulatory effect on TNFα macrophage production. An important role for the stimulation of IL-1β production was found of TLR2 and TLR6 as well as important for macrophage release of IL-12 [64]. A few studies did not find a correlation between TLR2 polymorphism and TB susceptibility 65.

TL4 a member of pattern recognition receptors (PRRs) plays critical role in host immune response against TB infection. In the current investigations observed that the mean concentrations of TLR4 (ng/ml) among male and female of tuberculosis patients were (3.35 ± 2.03) (2.99 ± 1.41) respectivly, was higher than male and female of control group (1.09 ± 0.45) (1.00 ± 0.58) respectively, statistically the differences were highly significant. In these results that the concentration of TLR4 in TB male patients slightly more than females. That matched with the study of [66] which shown that in *Mycobacterium tuberculosis*, TLR4 recognizes the cell wall lipids, glycoproteins and antigens. The surface of TLR4 expression on lymphocytes in TB patients was also reported as much as that in healthy control persons in the apparent of expression of both TLR4 and TLR2. In addition studies from West Africa TLR4 is necessary to detect Gram-negative bacteria's endotoxins and has been associated with pulmonary TB [67,68,69].

References

1. Aderem, A. and Ulevitch, RJ. (2002). "Toll like receptor in the induction of the innate immune response". Nature; 406 (6797): 782-787
2. Aftab, R.; Farzana, A. and Rukhshan, K. (2009). "Detection of Mycobacterium tuberculosis in clinical samples by smear and culture". J. physiol. 5: 27-30.
3. agier, J.; Żelechowska, P.; Kozłowska, E., et al. (2016). "Expression of surface and intracellular Toll-like receptors by mature mast cells". 41 (4): 333–338.
4. Ahmad, S. (2010). "Pathogenesis, Immunology, and Diagnosis of Latent Mycobacterium tuberculosis Infection". J. Immun. 2011, Article ID 3814943, 17.
5. Ahmed, N. and Hasnain, S. (2011). "Molecular epidemiology of tuberculosis in India: Moving forward with a systems biology approach". Tuberculosis. 91 (5):

407–413.

6. Balasingham, S.V. ; Davidsen, T. ; Szpinda, I.; et al. (2009). "Molecular diagnostics in tuberculosis: basis and implications for therapy". Mol. Diagn. Ther. ; 13 (3): 137-151.
7. Chai, Q.; Lu, Z. and Liu, C.H. (2020). "Host defense mechanisms against Mycobacterium tuberculosis". Cell. Mol. Life Sci. 77: 1859–1878.
8. Chang, J.S.; Huggett, J.F.; Dheda, K.; et al. (2006). "Myobacterium tuberculosis induces selective upregulation of TLRs in the mononuclear leukocytes of patients with active pulmonary tuberculosis". J. Immunol. 176: 3010-3018.
9. Che, M.; Li, A.C.; Wang, Y.M.; et al. (2017). "Expressions of toll-like receptors 2 and 4, and relative cellular factors in hiv patients with tuberculosis infection". Tropical Journal of Pharmaceutical Research, 16 (9): 2255–2259.
10. Dereje, A.; Bineyam, T.; Mohammad, A.; et al. (2012). "Epidemiology of antituberculosis drug resistance patterns and trends in tuberculosis referral hospital in Addis Ababa". Ethiopia. BMC Research Notes. 5: 462-470.
11. Gopalakrishnan, A.; Dietzold, J.; Verma, S.; et al. (2019). "Toll-like receptor 2 prevents neutrophildriven immunopathology during infection with mycobacterium tuberculosis by curtailing CXCL5 production". Infect. Immun.

87 (3): e00760-1.

12. Griebel P.J.; Brownlie R.; Manuja A.; et al. (2005). "Bovine toll-like receptor 9:

a comparative analysis of molecular structure, function and expression". Vet Immunol Immunopathol.;108 (1–2): 11–16.

13. Gringhuis; Dunnen, J.; Litjens, M.; et al. (2009). "Geijtenbeek. "TB. Carbohydrate-specific signaling through the DC-SIGN signal some tailors immunity to Mycobacterium tuberculosis, HIV-1 and Helicobacter pylori". J. Nat. Immun. 10: 1081–88.
14. Hasan, U.; Chaffois, C.; Gaillard, C.; et al. (2005). "Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88". Journal of immunology, 174 (5):

2942–2950.

15. Heil, F.; Hemmi, H.; Hochrein, H.; et al. (2004). "Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8". Science, 303 (5663): 1526-1529.
16. Hemmi, H.; Takeuchi, O.; Kawai, T.; et al. 2000. "A Toll-like Receptor Recognizes Bacterial DNA". Nature , 408: 740–745.
17. Huang, L.Y.; Ishii, K.J.; Akira, S.; et al. (2005). "Th1-like cytokine induction by heat-killed Brucella abortus is dependent on triggering of TLR9". J Immunol 175: 3964–3970.
18. Khan, N.; Pahari, S.; Vidyarthi, A.; et al. (2016). "NOD-2 and TLR-4 signaling reinforces the efficacy of dendritic cells and reduces the dose of TB drugs against Mycobacterium tuberculosis". J Innate Immun.;8 (3):228–242.
19. Lim, K.H. and Staudt, L.M. (2013). Toll-like receptor signaling. Cold Spring Harb. Perspect Biol. 5: a011247.
20. Liu, C.H., Liu, H. and Ge, B. (2017). "Innate immunity in tuberculosis: host defense vs pathogen evasion". Cell. Mol. Immunol. 14, 963–975.
21. Oberg, H.H. et al. (2010). "Differential but direct abolishment of human regulatory T cell suppressive capacity by various TLR2 ligands". J Immunol 184: 4733–4740.
22. O'Neill, L.A. (2008). The interleukin 1 receptor/Toll like receptor superfamily: 10 years of progress. Immunological reviews, 226 (1): 10-18.
23. ALSAIMARY, IHSAN E .A STUDY OF BACTRIAL PATHOGENES OF URINARY TRACT INFECTION (U.T.IS). AL-MUSTANSIRIAH J.SCI. 9(2):

41-48-1998.

24. IHSAN EDAN AL-SAIMARY, SUNDIS S. BAKR, KHALIL E. AL-HAMDI. STAPHYLOCOCCUS AUREUS AS A CAUSATIVE AGENT OF ATOPIC DERMATITIS/ ECZEMA SYNDROME (ADES) AND ITS THERAPUTIC IMPLICATIONS. ADVANCES IN BIORESEARCH . VOLUME 4 [1]

MARCH2013: 116 – 120 .

25. IHSAN EDAN AL-SAIMARY, KHALIL E. AL-HAMDI, SUNDIS S. BAKR . THE PREVALENCE OF ATOPIC ECZEMA / DERMATITIS SYNDROME (AEDS) IN BASRAH PROVIDENCE, IRAQ. ADVANCES IN BIORESEARCH. VOLUME 4 [1] MARCH 2013:126 - 129.
26. IHSAN E. AL-SAIMARY ,SUNDIS S. BAKR ,KHALIL E. ALHAMDI. BACTERIAL SKIN COLONIZATION IN PATIENTS WITH ATOPIC DERMATITIS/ ECZEMA SYNDROME. MEDICAL JOURNAL OF

ISLAMIC WORLD ACADEMY OF SCIENCES 21:4, 173-178, 2013

27. AL-SAIMARY IHSAN E.BACTERIAL WOUND INFECTIONS IN DIABETIC PATIENTS AND THEIR THERAPEUTIC IMPLICATIOBNS. MEDICAL PRACTICE AND REVIEW. (2010). VOL. 1 .NO.2 :12-15
28. AL-SAIMARY IHSAN E. EFFICACY OF SOME ANTIBACTERIAL AGENTS ON STAPHYLOCOCCUS AUREUS ISOLATED FROM VARIOUS BURN CASES. INTERNATIONAL JOURNAL OF MEDICINE AND
MEDICAL SCIENCES .(2009) VOL. 1 NO. 4: 110-114.
29. ALSAIMARY I, ET AL.CLINICAL FINDINGS AND PREVALENCE OF
HELICOBACTER PYLORI IN PATIENTS WITH GASTRITIS B IN ALBASRAH GOVERNORATE . OMAN MEDICAL JOURNAL. 24, 208-211
(2009).
30. AL-SAIMARY IHSAN E., AL-SHEMARI MAANI N. AND AL-FAYADH MOHAMMED M. A. EPIDEMIOLOGICAL AND IMMUNOLOGICAL FINDINGS ON HUMAN HYDATIDOSIS. AFRICAN JOURNAL OF
MICROBIOLOGY RESEARCHES. (2010) , 4(13) .
31. NIBRAS S. AL-AMMAR, SAAD SH. HAMADI, IHSAN AL-SAIMARY. DEMOGRAPHICAL STUDY OF H. PYLORI ASSOCIATED GASTRITIS . ADVANCES IN BIORESEARCH, VOL. 2 [1] JUNE 2011: 47- 61
32. Austin, J.F.; Dick J.M.; *et al.* (2004). "Gender Disparity Amongst TB Suspects and New TB Patients According to Data Recorded at the South African Institute of Medical Research Laboratory for the Western Cape Region of South Africa". International Journal of Tuberculosis and Lung Disease; 8 (4): 435–39. available at 5:30 pm in 4 July 2021.
33. Austin, J.F.; Dick J.M.; et al. (2004). "Gender Disparity Amongst TB Suspects and New TB Patients According to Data Recorded at the South African Institute of Medical Research Laboratory for the Western Cape Region of South Africa". International Journal of Tuberculosis and Lung Disease; 8 (4): 435–39. available at 5:30 pm in 4 July 2021.
34. Begum, V.; Colombani P. de.; Das Gupta, S.; et al. (2001). "Tuberculosis and patient gender in Bangladesh: sex differences in diagnosis and treatment outcome". Int J Tuberc Lung Dis; 5: 604-10.
35. Behera, D. (2010). Textbook of "Pulmonary Medicine" (2nd ed.). New Delhi: Jaypee Brothers Medical Pub. p.457.
36. Berrington W.R. and Hawn T.R. (2007). "Mycobacterium tuberculosis, macrophages and the innate immune response": does common variation matter? Immunol. Rev. 219: 167-186.
37. Blanc, L.; Gilleron, M.; Prandi, J.; et al. (2017). "Mycobacterium tuberculosis inhibits human innate immune responses via the production of TLR2 antagonist glycolipids". Proc. Natl. Acad. Sci. USA 114: 11205-11210.
38. Blumberg, H.M.; Burman, W.J. and Chaisson, R E. (2003). "American Thoracic Society/Centers for Disease Control and Prevention/ Infectious Diseases Society of America: treatment of tuberculosis". Am. J. Respir. Crit. Care Med. 167: 603–662.
39. Bodnar, K.A.; Serbina, N.V. and Flynn, J.L. (2001). "Fate of Mycobacterium tuberculosis within murine dendritic cells". J. Inf. and Immun. 69 : 800–809.
40. Khatri, G.R. and Frieden T.R. (2000). "The status and prospectus of tuberculosis control in India". Int J Tuberc Lung Dis;4:193-200
41. Pieters, J. (2008). "Mycobacterium tuberculosis and the Macrophage:
Maintaining a Balance". Cell Host & Microbe, 3(6), 399–407.
42. Powell, D.A. and Hunt, W.G. (2006). "Tuberculosis in children". J. Adv.
Pediatr, 53: 279–322.
43. Rahman, M.A.; Sobia, P.; Gupta, N.; et al. (2014). "Mycobacterium tuberculosis Subverts the TLR-2 - MyD88 Pathway to Facilitate Its Translocation into the Cytosol". PloS One; 9 (1): e86886.
44. Saqib, S.E., and Ahmad, M.M. (2018). "Development in Practice Socioeconomic determinants of the family history of pulmonary tuberculosis patients in Pakistan". Development in Practice, 0 (0): 1–12.
45. Small, P. M. and Pai, M. (2010)."Tuberculosis diagnosis - time for a game change" N. Engl. J. Med. 363: 1070-1071.
46. Thanky, N.R.; Young, D.B.; and Robertson, B.D. (2007). "Unusual features of the cell cycle in Mycobacteria: Polar –restricted growth and the snapping – model of cell division". J. Tubercle. 87 (3): 231-236.
47. Velayati, A.A.; Farnia, P.; Masjedi, M.R.; et al.(2011). "Sequential adaptation in latent tuberculosis bacilli: observation by atomic force microscopy". J. Clin.
Exp. Med.4: 124-149.
48. Behera, D. (2010). Textbook of "Pulmonary Medicine" (2nd ed.). New Delhi: Jaypee Brothers Medical Pub. p.457.
49. Berrington W.R. and Hawn T.R. (2007). "Mycobacterium tuberculosis, macrophages and the innate immune response": does common variation matter? Immunol. Rev. 219: 167-186.
50. Blanc, L.; Gilleron, M.; Prandi, J.; *et al*. (2017). "Mycobacterium tuberculosis inhibits human innate immune responses via the production of TLR2 antagonist glycolipids". Proc. Natl. Acad. Sci. USA 114: 11205-11210.
51. Blumberg, H.M.; Burman, W.J. and Chaisson, R E. (2003). "American Thoracic Society/Centers for

Disease Control and Prevention/ Infectious Diseases Society of America: treatment of tuberculosis". Am. J. Respir. Crit. Care Med. 167: 603–662.

52. Falih hmood mezban, Ihsan edan alsaimary. 2016. Significance of Skin test reactivity to aeroallergens in patients with chest and skin allergies in basrah through 2014 and 2015. Basic Research Journal of Microbiology. Vol. 3(6)
53. Maha M. Al-mahfoud, Ihsan E. AlSaimary and Ali. A. Al shawi. Occurrence of oral and oropharyngeal squamous cell carcinoma among patients in Basrah city, : Journal of Physics: Conf. Series 1279 (2019) 012074 .IOP Publishing .doi:10.1088/1742-6596/1279/1/01207470
54. Farqad M. Al-Hamdani, , Ihsan E. AlSaimary and Khalil I. Al Hamdi. Molecular characterization of Malassezia spp isolated from human pityriasis versicolor. Medico-legal Update, July-December 2019, Vol.19, No. 2
55. Ban A Alkhafaji and Ihsan Edan Alsaimary. Comparative Molecular Analysis of Meca, Sea and Seb Genes in Methicillin-Resistant Staphylococcus Aureus

(MRSA). J Biotechnol Bioinforma Res, 2020. Volume 2(3): 2-8

56. ALMusafer, Murtadha M. Hussein N. AlDhaheri Dr.Ihsan Edan A. AlSaimary
57. Clinical study of patients with prostatitis in Basrah and Missan
58. governments: a case –control study . Journal of Medical Research and Health Sciences. 3 (11), 1110-1115 (2020)
59. AlSaimary, Ihsan Edan and Falih Hmood Mezban. Clinical findings of patients with human bronchial asthma in Basrah , Iraq. Applied medical research.8(2).:1-4. 2021. ISSN: 2149-2018
60. DOI: 10.5455/amr.20211040 .
61. Bodnar, K.A.; Serbina, N.V. and Flynn, J.L. (2001). "Fate of *Mycobacterium tuberculosis* within murine dendritic cells". J. Inf. and Immun. 69 : 800–809.
62. Khatri, G.R. and Frieden T.R. (2000). "The status and prospectus of tuberculosis control in India". Int J Tuberc Lung Dis;4:193-200
63. Pieters, J. (2008). "Mycobacterium tuberculosis and the Macrophage:

Maintaining a Balance". Cell Host & Microbe, 3(6), 399–407.

64. Powell, D.A. and Hunt, W.G. (2006). "Tuberculosis in children". J. Adv. Pediatr, 53: 279–322.
65. Rahman, M.A.; Sobia, P.; Gupta, N.; *et al.* (2014). "Mycobacterium tuberculosis Subverts the TLR-2 - MyD88 Pathway to Facilitate Its Translocation into the Cytosol". PloS One; 9 (1): e86886.
66. Saqib, S.E., and Ahmad, M.M. (2018). "Development in Practice Socioeconomic determinants of the family history of pulmonary tuberculosis patients in Pakistan". Development in Practice, 0 (0): 1–12.
67. Small, P. M. and Pai, M. (2010)."Tuberculosis diagnosis - time for a game change" N. Engl. J. Med. 363: 1070-1071.
68. Thanky, N.R.; Young, D.B.; and Robertson, B.D. (2007). "Unusual features of the cell cycle in Mycobacteria: Polar –restricted growth and the snapping – model of cell division". J. Tubercle. 87 (3): 231-236.
69. Velayati, A.A.; Farnia, P.; Masjedi, M.R.; *et al.(*2011). "Sequential adaptation in latent tuberculosis bacilli: observation by atomic force microscopy". J. Clin.

Exp. Med.4: 124-149.