Open Access
Baghdad Science Journal **Vol.16(1)Supplement 2019**

DOI: http://dx.doi.org/10.21123/bsj.2019.16.1(Suppl.).0178

DNA Methylation Patterns of Interferon Gamma Gene Promoter and Serum Level in Pulmonary Tuberculosis: Their Role in Prognosis

Fawzi Hassan Zayr*[1]** ***Maysaa A. R. Dhahi*[2] ***Omar F. Abdul-Rasheed*[3]**
***Hashim M. Hashim*[4]**

Received 3/6/2018, Accepted 23/1/2019, Published 17/3/2019

Abstract:

Tuberculosis (TB) still remains an important medical problem due to high levels of morbidity and mortality worldwide. A series of innate immune mechanisms that create a cytokine network control the pathogenesis of tuberculosis and this response has the capacity to modify the host genomic DNA structure through epigenetic mechanisms such as DNA methylation which could constantly alter the local gene expression pattern that can modulate the metabolism of the tissues and the immune-response. Interferon-gamma (IFN-γ) is an important pro-inflammatory cytokine regulator of the innate immune response to TB. This study aims to determine DNA methylation patterns of INF-γ gene promoter and measure serum IFN- γ level in newly diagnosed TB patients, relapse TB patients, and healthy control, in order to study the possibility of using these as a biomarker for the prognosis of TB stages in patients. The current case-control study included 66 patients with TB and 33 healthy control subjects. DNA was extracted from peripheral blood(PB) of included subjects and modified using sodium bisulfate specific kit. DNA methylation patterns of IFN-γ gene promoter was determine by using methylation specific polymerase chain reaction(MS-PCR).Serum IFN-γ level was determined using enzyme linked immune-sorbent assay(ELISA). Results showed that percentages of DNA methylation patterns in normal controls, newly diagnostic TB patients and relapse TB patients were (63.3%, 18.2% and 21.2% respectively). Also, higher significant differences (P≤0.0001) of un-methylated IFN-γ gene promoter patterns in newly diagnostic TB patients than relapse TB patients comparison with healthy controls. The percentage of un-methylated DNA patterns in healthy controls, newly diagnostic TB patients and relapse TB patients were (9.9%, 39.4% and 51.5%, respectively). The mean of serum IFN-γ levels (pg/ml) for normal controls, newly diagnostic TB patients and relapse TB patients were (59.3± 13.8,75.8±24.3 and 69.6±18.7,respectively).In conclusion, there is a relative association between methylation of IFN-γ gene promoter and predisposing to TB progression.

Key words: Disease progression, Interferon gamma gene promoter DNA methylation, Interferon gamma level, *Mycobacterium tuberculosis*.

Introduction:

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and it remains one of the world's biggest threats. In 2014, TB killed 1.5 million people (1.1 million HIV-negative and 0.4 million HIV-positive) (1,2). The World Health Organization (WHO) suggested new strategy that set targets for TB elimination for the first time in 2015.

To achieve these ambitious goals, new drugs, new diagnostics methods and vaccines will be needed (3). Molecular epidemioloical studies referred that the development of active TB was due to reactivation of the original strain that may occur decades after the initial infection (4). The development of infection from latent TB (LTB) to primery TB (PTB) reflects deficincy of host resistance to *M. tuberculosis* (5). There is a strong evidence that the development of LTB is controled by host genetic factors.These genetic factores are likely to be different from these involved in PTB(6).

During infection with *M. tuberculosis*, the host innate immune response composes the first defense,then bacteria will be taken up by the reside phagocytic cells in the lungs (7,8). Interferon-gamma (IFN-γ, also known as type II interferon) is

[1] Department of Chemistry and Biochemistry, College of Medicine-Wasit University, Wasit, Iraq.
[2] Department of Medical Microbiology College of Medicine-Al-Nahrain University, Baghdad, Iraq.
[3] Department of Chemistry and Biochemistry, College of Medicine-Al-Nahrain University, Baghdad, Iraq.
[4] Department of Medicine, College of Medicine-Al-Nahrain University, Baghdad, Iraq.
*Corresponding author: dr_maysaa@yahoo.com.

an important immune-regulatory cytokine that was originally identified as an anti-viral agent(9). It plays a key role in host defense by exerting anti-proliferative and immune regulatory activities (8).The mycobacterial TB has the capacity to alter the host DNA structure through epigenetic mechanisms. Epigenetics is defined as an alteration in conformational structure of DNA without any changes in the sequence that result in making it more or less accessible to transcription process. DNA methylation is considered a major epigenetic alteration in human DNA which change the local gene expression by inducing genomic-epigenetic changes that can affect the metabolism of the tissues and the inflammatory response(10).

Human immune response such as cytokine production, anti-inflammatory response and cell differentiation regulated by DNA methylation, in addition to genetic control. In eukaryotes, methyl group(CH_3) added to the position of carbon number 5 of the cytosine in CpG islands. Un-methylated islands are related with actively transcripted gene, whereas methylated islands can bind with methyl-binding proteins that up hold chromatin compaction and avoid the binding of transcription factors to these dinucleotide. Variations in the epigenome, and gene polymorphism of different ethnic groups control host susceptibility to TB infection(11). This epigenetic alteration may explain the host-pathogen interaction of *M. tuberculosis* and act as a bio-markers that decide what situation will be latency or disease(12).

The cytokine networks established by the macrophages in the innate immune response play a critical role in the control of *M. tuberculosis* infection, but the underlying mechanism for *M. tuberculosis* infection to induce hyper-methylation of cytokine remains poorly understood(13).The current study aims to investigate the utility of DNA methylation patterns of INF-γ gene promoter and INF- γ serum level in TB patients, in order to determine the usefulness of these biomarkers as early prognosis tool in pulmonary tuberculosis.

Materials and Methods:

Patients

The current case-control study included 66 samples from patients with TB collected during period from April 2016 to April 2017. Also,33 samples from healthy controls were included. Patients age ranged from 16-70years and healthy controls age range from 20-65 years. Peripheral blood samples were collected from enrolled patients whom attend TB Centers in Baghdad, Wasit and Al-Emamen Al-Kadhmain Teaching Hospital. Samples from patients(66 samples) and healthy controls (33 samples) were taken from both genders (54 male and 45 female).The subjects included in this study were grouped as following:

Group 1: included 33 newly diagnosed patients with active PTB whom did not receive anti-tuberculosis treatment before sampling. Those patients had been re-evaluated after 2 months of anti-TB therapy. Serum IFN-γ level was performed before treatment and after 2 months of patient therapy (rifampicin + isoniazide + ethambutol and pyrazinamide), when their sputum became negative for acid fast bacilli (AFB).

Group 2: included 33 relapsed cases of PTB. Those patients had been diagnosed with TB and whom had completed the treatment successfully but was diagnosed with TB again. That was managed by AFB smear positivity in sputum sample or those with both cavitation on initial chest radiography and positive sputum smear after 1-2 years of anti-TB treatment.

Group 3: included 33 healthy adults. All healthy volunteers were checked for the chronic and common disease such as diabetes mellitus, renal failure or heart disease and no history or signs of active PTB and also negative for AFB.

Inclusion criteria specified consideration of a TB dataset from any of the following active medical condition will be excluded : HIV infection, nephritic syndrome, enteric fever, viral hepatitis, diabetes mellitus, leprosy, infectious mononucleosis, chronic malnutrition, and Asthma.
This study was approved by the ethical committee of the College of Medicine-Al-Nahrain University, Baghdad, Iraq.

Samples

Ten milliliters of venous blood were drawn from each subject and preserved in two tube,(5 ml) in an anti-coagulated tubes containing sodium-EDTA, and (5 ml) in the second plain tube for serum and sera will be separated by centrifugation at 1500xg for 10 min at 4◦C, divided into small aliquots and stored at -80◦C until used.

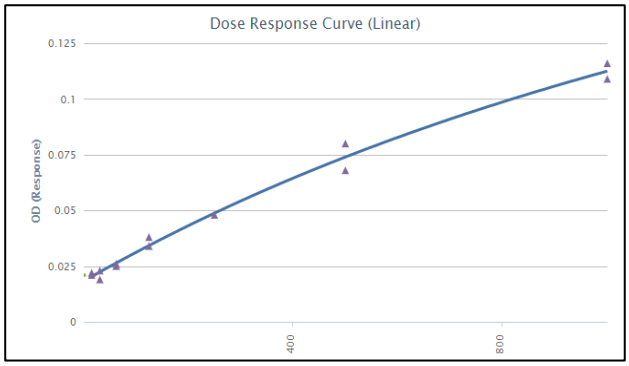
Identification of *M. tuberculosis* in Patient Sputum Sample

Acid-Fast Staining of direct sputum smear was prepared (14).The smear was examined microscopically by specialist in hospital and center lab, using the 100X oil immersion objective.

Quantitative Determination of IFN-γ Serum Level Using Sandwich Enzyme Immunoassay Technique

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human IFN-γ (R&D system a biotechne brand catalog number

DIF50, USA) was used following manufacturer instructions and was done in ELISA system (biotek, USA). Briefly, a polyclonal antibody specific for human IFN-γ had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any IFN-γ present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IFN-γ was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IFN-γ bound in the initial step. The color development was stopped and the intensity of the color was measured. The optical density of each well was determined after within 30 min by using a microplate reader set to 450 nm, Fig.1.



Concentration of IFN-γ (pg/ml)

Figure 1. Standard curve of IFN-ɣ concentration

Determination of DNA Methylation Patterns of IFN-γ Gene Promoter Using MS-PCR

DNA Extraction

Genomic DNA was extracted from PB samples using QIAamp DNA Mini kit(Qiagen, USA) following manufacturer instructions. Purity and concentration of DNA samples were measured using spectrophotometer (Apel303uv-Japan).

Sodium Bisulfate Modification

DNA methylation status was modified using sodium bisulfite modification kit (Qiagen, USA) following manufacturer instructions which includes incubation of the target DNA with sodium bisulfite to convert un-methylated cytosine into uracil while it leaves the methylated cytosines unchanged. Therefore, bisulfite treatment gives differences between methylated DNA sequences and un-methylated DNA sequences.

Methylation Specific PCR (MS-PCR) for Detection of DNA Methylation Patterns of IFN-γ Gene Promoter

Methylation patterns of IFN-γ gene promoter was detected in modified DNA samples included in the present study using MS-PCR(12).Two primer sets, one for methylated state and the other for non-methylated state of IFN- γ gene promoter were used. The amplified products were electrophoresed separately on 2% agarose gel. Presence of band with 154 bp means presence of methylated state, while the presence of band with 156 bp by using the primer for detection of un-methylation of IFN-γ gene promoter means there was no methylation. If both of the above bands were presented, that means partial methylation of IFN-γ gene promoter.

Thermal cycler (LABNET,USA) was used. Optimization of reaction conditions was done by using different concentration of primers (1µl, 1.5µl and 2µl), different concentrations of DNA were (2µl,3 µl, and 5 µl) and gradient annealing temperature was from (48-69)°C.

Determination of Serum INF- γ Level

Receiver operating characteristics (ROC) curves were applied to compare the performance of the biochemical prognostic methods of disease and to determine the appropriate cut off values of serum IFN-γ level in TB patient compared with normal control by using 59.3 pg/ml as cut-off value. The optimal cut-off value for serum IFN-γ level in all groups estimated from ROC curves, according to these results, test is considered positive if test is > threshold value (cut off values).

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to analyze the prognostic value of serum INF-γ level and they were 75.5%, 60%, 79.4% and 55.6%,respictivly.

Statistical Analysis:

Chi-square test used to compare frequency. Chi- square test was performed for methylation and un-methylation of IFN-γ gene promoter to determine the significant of the test(15).

Results:

Serum IFN-γ Level

Serum IFN-γ level was measured to evaluate its usefulness in prognosis and follow up of patients with TB disease. Table 1 shows the mean ±SD of serum IFN-γ level expressed as pg/ml in sera of normal controls (59.3± 13.8), newly diagnosis TB patients (75.8±24.3), and relapse TB patients (69.6±18.7).

Table1. Serum IFN- γ level in normal controls, newly diagnostic TB patients and relapse TB patients (± SD).

IFN-γ (pg/ml)	Normal control	TB patients	Relapse patients
Mean ±SD	59.3± 13.8	75.8±24.3	69.6±18.7
Range	44–123	49–160	51–140

The bio-statistical calculation and studies t-test were done by using SPSS program (version 22) for IFN-γ level in sera of normal controls, newly diagnostic TB patients and relapse TB patients. The Serum IFN-γ level from both newly diagnose TB patients and relapse TB patients were high significantly increased (P<0.001 and 0.014, respectively) when compared to normal controls.

Determination of DNA Methylation Patterns of IFN-γ Gene Promoter Using Methylation Specific-PCR (MSP)

The analyses of the differentially DNA methylated patterns of IFN-γ gene promoter were compared between patients with active TB disease and normal controls subjects or between relapsed TB patients and normal controls subjects. Methylation specific-PCR results of IFN-γ gene promoter of included patients and controls showed in Table 2 .

Table 2. Percentages of DNA methylation pattern of IFN-γ gene promoter of studied subjects.

Groups	No. of subjects have IFN-γ methylated gene promoter	Percentage%	No. of subjects have IFN-γ un-methylated gene promoter	Percentage%	No. of subjects have IFN-γ partial methylated gene promoter *	Percentage%	Total cases
Healthy control	21	63.3%	3	9.9%	9	27.3%	33
Newly diagnostic TB	6	18.2%	13	39.4%	14	42.4%	33
Relapse TB	7	21.2%	17	51.5%	9	27.7%	33

* Partial methylated gene: the appearance of PCR amplified bands in both states of using DNA methylation detection primers(154 bp) and DNA un-methylation detection primers (156bp) for the same modified DNA sample.

Agarose gel electrophoresis of MSP-PCR amplified products in different studied groups were shown in Fig. 2 . Samples were defined as methylated and un-methylated depending upon the visual band that amplified using primer set to detect methylated (M) or un-methylated (U) sites.

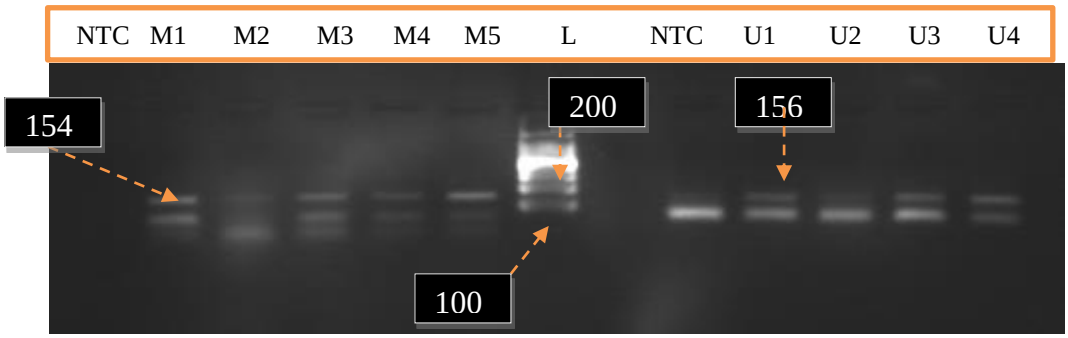


Figure 2. Agarose gel electrophoresis demonstrating the MSP-PCR results of IFN-γ gene promoter.

Lane M (1-5):Amplified products of sodium bisulfate modified DNA were extracted from patients (1-5) using DNA methylation detection primer set. Lane U(1-5): Amplified products of sodium bisulfate modified DNA were extracted from patients (1-5) using DNA un-methylation detection primer set. Lane (L): DNA ladder of 100bp. Lane (NTC):No template control. Electrophoresis was done on 2.5% agarose, at 70 V and for 60 min. IFN-γ gene promote in patients (1,3):methylated. IFN-γ gene promote in patient(2): un-methylated. IFN-γ gene promote in patients (4,5): partial methylated.

Chi-Square test and P-Value for DNA methylated patterns of IFN-γ gene promoter in normal controls, newly diagnose TB patients and relapse TB patients were calculated. It showed high significant increase (P≤0.002 and P≤0.001, respectively) for DNA methylated patterns of IFN-γ gene promoter in normal controls, newly diagnostic TB patients and relapse TB patients. Also, it showed high significant increase (P≤0.0001 and

P≤0.0001, respectively) for DNA un-methylated pattern of IFN-γ gene promoter in normal controls, newly diagnostic TB patients and relapse TB patients. That referred to the possibility of use DNA methylation patterns of IFN- γ gene promoter as prognostic marker in TB patients.

Discussion:

Serum IFN-γ level

A number of signaling pathways control the initial infection of the macrophages with the bacteria elevates the host inflammatory response (16). Pro-inflammatory cytokines including IFN-γ are produced from activated macrophages as response to toll like receptor-2(TLR-2) signaling pathway. In active tuberculosis, serum levels of these mediators and IFN-γ levels have been proved high in patients (11).Interferon gamma is one of the most important pro-inflammatory cytokines in many infectious or noninfectious chronic inflammatory diseases, and whole blood IFN-*γ* assays are popular in diagnosis of human tuberculosis (17, 18, 19).

In the current study, the results revealed highly significant elevation in serum IFN-γ level(Table 1) in newly diagnostic TB patients, relapse TB patients and healthy controls (P<0.001and P<0.014, respectively). These results are consistent with previous studies showing elevated plasma levels of IFN-*γ* in newly diagnosed TB patients and relapse TB patients suggesting up-regulation in the pro-inflammatory response(18, 19).

The criteria of diagnosis validity (sensitivity, specificity, positive predictability, negative predictability and efficiency tests) of serum IFN-γ level in TB patients compared to that of healthy controls, using 59.3 pg/ml as cut-off value (clinical decision),were 75.5%, 60%, 79.4%, 55.4%,and 70.7%, respectively. The fact that serum IFN-γ levels are up-regulated in the newly diagnosed TB patients indicates innate protective response during early phase of *M. tuberculosis* infection. The results of the current study agree with previous studies (19-23).These findings could be referred to possibility of developing immune-prognostic assays based on new biomarkers detection.

DNA Methylation Pattern of IFN-γ Gene Promoter

Mycobacterium tuberculosis may be drip the bactericidal effects of phagosomes multiply and survive in the infected host cells through activating an anti-inflammatory response and struggling acidification of the phagosomes by enhancing an adaptive response with T-cell activation and further triggering of macrophages and neutrophil accumulation to form granulomas leading to latent infections (LTBI) that may become active tuberculosis only as the host's immune response fails(11, 24).

Mycobacteria can alter the methylation of host gene to modify the host immune system situations (25).The dynamic nature of the epigenome enable cells to translate environmental signals to appropriate response by modifying its transcriptional machinery (26, 27). Modification in DNA methylation during *M. tuberculosis* infection was seen across the whole macrophage genome. It was referred to correlation between methylation status of gene body(the rest of gene sequences other than promoter sequence) gene regulation but most studies showed a strong association of gene promoter methylation with gene transcription (28). However, DNA methylation modifications upon *M. tuberculosis* infection mostly associated with non-promoter, non-exonic regions of the genome. Tissue-specific-methylated non-promoter regions are cis-regulatory elements like enhancers, transcriptional activators or repressors, so, regulatory regions were targeted by *M. tuberculosis* during infection of macrophages and that finding was also confirmed by several non-coding RNA genes, which have role in the regulation of gene expression, were the targets of infection agents induced DNA methylation modification(25, 29-31).

In the current study, percentages of DNA methylation, partial methylation, and un-methylated patterns of IFN-γ gene promoter were determined in different studied groups. In the health controls, 63.5% of the samples showed total methylation of IFN-γ gene promoter, 9.5% showed un-methylated of IFN-γ gene promoter and 27% showed partial methylation. In newly diagnostic TB patients, 18.2% of the samples showed total methylation of IFN-γ gene promoter,39.4% positive for un-methylated of IFN-γ gene promoter and 42.4% showed partial methylation of IFN-γ gene promoter and in relapse TB patients, 21.2% of the samples showed total methylation of IFN-γ gene promoter,51.5% positive un-methylated of IFN-γ gene promoter and 27.3% showed partial methylation. The results tend to decrease in methylation or hypo-methylation in IFN-γ gene promoter in patients with TB and increase in un-methylation form. These are consider of switch on of DNA expression for IFN-γ gene promoter.

There are confirmed information regarding DNA methylation of IFN-γ promoter, but investigation of status of other relevant sequences for IFN-*γ* gene expression in lymphocytes need more studies. Also, the role of other epigenetic marks, such as histone modifications and micro-RNA, in pathogenesis patterns of *M. tuberculosis* need to be investigated.

Conclusion:

The current study identified significant DNA methylation alterations in the IFN-γ gene promoter in human peripheral blood from TB patients. Methylated and un-methylated status changes of IFN-γ gene promoter were significant in active TB infection, which also supported this point. Further studies on the epigenetic changes of IFN-γ gene associated with various states of TB infection will enable further understanding of their functional role in the response to TB.

Conflicts of Interest: None.

References:

1. Global tuberculosis report WHO Library Cataloguing-in-Publication Data, 2018:6.
2. Garnaut J.Centers for Disease Control and Prevention. History of world TB day.2012;8: 2229-2232.
3. Sinh TT. Tuberculosis in Children : Diagnosis and Epidemiology. New York: Oxford University Press; 2017.
4. Savolainen LE. Characterization of diagnostic biomarkers for *Mycobacterium tuberculosis* infection. Finland: University of Helsinki Helsinki, Finland, Thesis 2014.
5. Sector P. Vitamins D receptor polymorphism & Active tuberculosis. Indian J. Tuberc.2013;60(4):3–6.
6. Driver ER. The C3HeB/FeJ mice as a novel preclinical mouse model for *Mycobacterium tuberculosis* : an analysis of the host pathogenesis and the *in vivo* environment of the necrotic granuloma.Thesis:2014.
7. Smith JA. Fuctional analysis of ESAT-6 and ESPB, two virulence proteins secreted by the ESX-1 system in mycobecrium marinum. Thesis,2010.
8. Obregon-Henao A, "Novel extracellular products of Mycobacterium tuberculosis: Composition, synthesis, and relevance to disease. Thesis, 2013.
9. Cavalcanti YVN, Brelaz MCA, Neves DAL,Ferraz JC. Role of TNF-alpha, IFN-gamma, and IL-10 in the development of pulmonary tuberculosis. Pulm. Med.2012; 2012.
10. Cardoso F. Methylation Pattern of the IFN- γ Gene in Human Dental Pulp. J. Endod.2012;36(4): 642–646.
11. Zheng L, Leung ETY, Wong HK, G. Lui, Lee N,To KF,et al. Unraveling methylation changes of host macrophages in *Mycobacterium tuberculosis* infection. Tuberculosis.2016; 98:139–148.
12. Dong M, Chunyang J, Xiaoli H, Qingzhao LI, Tingting LI, Yanyan Y, et al. Methylation patterns of the IFN-gamma gene in cervical cancer tissues. Science reports, 2014.
13. Walker DM, Gore AC. Epigenetic impacts of endocrine disruptors in the brain. Front. Neuroendocrinol.2017;44:1–26.
14. Marais BJ. Tuberculosis in children. J. Paediatr Child Health.2014;50(10):759-67.
15. Ganapathi1 SK, Beggs AD, Hodgson SV ,Kumar DK. Expression and DNA methylation of TNF, IFNG and FOXP3 in colorectal cancer and their prognostic significance. British J. of Cancer.2014; 111:1581–1589.
16. Azad AK, Sadee W, Schlesingera LS. Innate Immune Gene Polymorphisms in Tuberculosis. Infection and Immunity.2012;80(10):3343–3359.
17. Hungria EM, Freitas AA, Pontes MA, Gonçalves HS, Sousa AL, Costa MB,et al. Antigen-specific secretion of IFNγ and CXCL10 in whole blood assay detects *Mycobacterium leprae* infection but does not discriminate asymptomatic infection from symptomatic leprosy. Diagn. Microbiol. Infect. Dis.2017;87(4):328–334.
18. Villar-Hernández R, Latorre I, Mínguez S, Díaz J, García-García E, Muriel-Moreno B,et al. Use of IFN-γ and IP-10 detection in the diagnosis of latent tuberculosis infection in patients with inflammatory rheumatic diseases. J. Infect.2017; 75(4):315–325.
19. Min F, Wu R, Pan J, Huang SH, Luo Y, Zhang Y. Positive Correlation between IP-10 and IFN-γ Levels in Rhesus Monkeys (*Macaca mulatta*) with either naturally acquired or experimental infection of *Mycobacterium tuberculosis*. Bio. Med. Res.Int.2017;2017(1):1–6.
20. Shaviya N, Budambula V, Webale MK,Were T. Circulating interferon-gamma levels are associated with low body weight in newly diagnosed kenyan non-substance using Tuberculosis individuals. Interdiscip. Perspect. Infect. Dis.2016;1-9.
21. Latorrei I, Díaz J, Mialdea I, Serra-Vidal M, Altet N, Prat C, et al.IP-10 is an accurate biomarker for the diagnosis of tuberculosis in children. J. Infect.2014;69(6):590–599.
22. You E, Kim MH, Lee WI, and Kang SY. Evaluation of IL-2, IL-10, IL-17 and IP-10 as potent discriminative markers for active tuberculosis among pulmonary tuberculosis suspects. Elsevier Ltd.2016;99:100-8.
23. Hong JY, Lee HJ, Kim SY, Chung KS, Kim EY, Jung JY, et al. Efficacy of IP-10 as a biomarker for monitoring tuberculosis treatment.J. Infect. 2014;68(3):252–258.
24. Yaseen I, Kaur P, Nandicoori VK, Khosla S. Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. Nat Commun.2015;6:8922.
25. Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK, Khosla S. The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. Nucleic Acids Res.2015;43(8):3922-37.
26. Jones PA. Functions of DNA methylation, islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 2012;13:484–492.
27. Maston GA, Evans SK,Green MR. Transcriptional regulatory elements in the human genome. Annu. Rev. Genomics Hum. Genet.2006;7:29–59.
28. Kaikkonen MU, Lam MT, Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc. Res.2011;90:430–440.

29. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, et al. Host directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk". Nature. 2014;511(7507):99-103.
30. Hur YG, Gorak-Stolinska P, Ben-Smith A, Lalor MK, Chaguluka S, Dacombe R, et al. Combination of cytokine responses indicative of latent TB and active TB in Malawian adults. PLoS One.2013; 8(11), e79742.
31. Wareham AS, Tree JA, Marsh PD, Butcher PD, Dennis M, Sharpe SA. Evidence for a role for interleukin-17, Th17 cells and iron homeostasis in protective immunity against tuberculosis in Cynomolgus Macaques. PLoS One. 2014; 9(2): e88149.

طرز مثيلة الدنا لمشغل جين الانترفيرون كاما ومستواه في المصل لمرضى التدرن الرئوي– دورهما في الاستعدادية للإصابة

فوزي حسن زاير [1] ميساء عبد الرزاق ضاحي [2] عمر فاروق عبد الرشيد [3] هاشم محمد هاشم[4]

[1]قسم الكيمياء، كلية الطب، جامعة واسط، واسط ، العراق.
[2]فرع الاحياء المجهرية الطبية ،كلية الطب، جامعة النهرين، بغداد، العراق.
[3]فرع الكيمياء والكيمياء الحياتية، كلية الطب، جامعة النهرين، بغداد، العراق.
[4]فرع الطب الباطني، كلية الطب، جامعة النهرين، بغداد، العراق.

الخلاصة:

يعد مرض التدرن الرئوي مشكلة صحية عالمية لما يسببه من إمراضيه ووفيات. تنشا سلسلة من الاستجابات المناعية الفطرية نتيجة الاصابة بالتدرن الرئوي والتي تتمثل بشبكة من الحركيات الخلوية للسيطرة على إمراضية التدرن الرئوي وهذه الاستجابة سوف تحور تركيبة دنا الخلايا المصابة من خلال اليات التخليق المتعاقب مثل مثيلة الدنا والذي من الممكن ان يؤدي الى تحوير في طرز التعبير الجيني وبالتالي يغير في العمليات الايضية النسيجية والاستجابة المناعية. يعد الانترفيرون كاما احد الحركيات الخلوية للاستجابة الالتهابية الأولية للتدرن الرئوي . تهدف الدراسة الحالية الى تحديد طرز مثيلة الدنا لمشغل جين الانترفيرون كاما وقياس مستوى الانترفيرون كاما في مصل الدم في مرضى التدرن الرئوي المشخصة اصابتهم حديثا و مرضى التدرن الرئوي المتكرر وكذلك السيطرة من الاشخاص الاصحاء وذلك لدراسة امكانية استخدامها كمعلمات بيولوجية في دراسة الاستعدادية للإصابة بالمرض. تم جمع 66 عينه دم من المرضى الذين يعانون من مرض التدرن الرئوي بواقع 33 عينة من مصابين تم تشخيصهم حديثا و33 عينة من مرضى التدرن الرئوي المتكرر للفترة من (1-4 - 2016 ولغاية 1- 4- 2017) و33 عينه دم من الاشخاص الاصحاء .تم الحصول على العينات المرضية من مركز التدرن في بغداد ومستشفى واسط ومستشفى الإمامين الكاظمين(ع).

تم قياس مستوي الانترفيرون كاما في مصل 66 مريضًا وكذلك مصل 33 من الاصحاء بطريقة التفاعل المناعي المقترن بالأنزيم. استخلص الدنا من عينات الدم المحيطي وتم تحوير تركيبته باستخدام عدة متخصصة من الصوديوم ثنائي الكبريت. تم الكشف عن طراز المثيلة في دنا مشغل جين الانترفيرون كاما للاشخاص قيد الدراسة بواسطة طرق تفاعل البلمرة المتسلسلة المختص بالمثيلة. اشارة النتائج الاحصائية الى وجود علاقة معنوية بين مثيلة مشغل جين الانترفيرون كاما للأشخاص الاصحاء و مرضى التدرن الرئوي حديثي التشخيص ومرضى التدرن الرئوي المتكرر وكانت النسب المئوية للدنا الممثيل (63.3% ،18.2% و 21.2%، على التوالي)، كذلك وجود علاقة احصائية معنوية للاختلافات في طرز الدنا الغير ممثيل بين المجاميع قيد الدراسة وكانت النسب المئوية للدنا الغير ممثيل (9.9% ،39.4% و51.5% ، على التوالي). اشارت النتائج الى وجود علاقة احصائية معنوية للاختلافات في متوسط مستوى الانترفيرون كاما (بيكوغرام/ مل) في مصل الأشخاص الاصحاء و مرضى التدرن الرئوي حديثي التشخيص ومرضى التدرن الرئوي المتكرر.يستنتج مما سبق وجود ترافق نسبي بين مثيلة مشغل جين الانترفيرون كاما والاستعدادية لتطور الاصابة بالتدرن الرئوي.

الكلمات المفتاحية: تطور المرض، مستوى الانترفيرون كاما، مثيلة مشغل جين الانترفيرون كاما ، *Mycobacterium tuberculosis*.