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Receptors (TLR2 , TLR4 and TLR9 } in gene bank – NCBI for patients with tuberculosis in Basrah - IRAQ

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Abstract

A case-control study has been carried out among patients with Tuberculosis,who attended to the Consultation Center of Chest and Respiratory diseases of Basrah province during1st September 2020 to 1st June 2021. From total number of (176) patients with Tuberculosis (TB) were taken from Basrah province that included in the present study. The molecular study include extract of DNA from blood of TB patients and control group by using specific primers in conventional PCR, the amplification of all extracted DNA from blood samples was preform and confirm by using electrophoresis with (70 volt / 60 minutes) on agarose gel (1%), the results of gene expression revealed that the amplified DNA(PCR product) was 154bp for TLR2 with presence ratio 85.2% from total samples, 143bp for TLR4 with a presence of 100.0%, and 177 bp for TLR9 with a presence ratio 98.8%.In the sequencing study the results from sequencing of TLR2,TLR4 and TLR9 were showed as follows: there were clear convergence between our TLR2 gene and that of the GenBank database (NCBI) with identity 109/114 (96%) in forward-TLR2 had five mutations appeared as G to A; T to A; --- to A; T to C and T to C. In addition, the reverse-TLR2 was shown an identity 106/108 (98%) in comparison with GenBank database (NCBI) that founded two mutations in reverse TLR2 as T to C and C to T. And there was a low convergence between our TLR4 genes and that of the GenBank database (NCBI) with identity 111/113 (98%) in forward-TLR4 that were recorded two mutations appeared as G to A and C to ---. In the other side the reverse TLR4 shown an identity 106/113 (94%) when compared with GenBank database (NCBI) seven mutations were reported as T to ---; T to ---; T to G; A to C; A to G; A to C and T to C. And there was a highly convergence between our TLR9 genes and that of the GenBank database (NCBI) in

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identity 132/134 (99%) that founded two mutations in the forward-TLR9 as --- to C and G to C. And for reverse-TLR9 showed no mutations when we compared with GenBank (NCBI) database shown identity 128/128 (100%), there are no studies interested with sequencing of TLRs with relation of tuberculosis so we will compare our results with studies on other diseases.

Key words: tuberculosis , , Basrah , Toll- Like Receptors(TLR) , gene ,DNA sequence , phylogenetic trre , gene bank , NCBI

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Introduction

Tuberculosing 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 role3,4,5. 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 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 pathogens11 , 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

TB also has several strategies to disturb these defenses, such as

- interference with phagosomal maturation and acidification ,
- resistance to oxidative stresses ,
- escape to the cytosol ,
- formation of granulomas ,
- modulation of host cell death 20
- And, TB can inhibit host innate immune systems by producing cellular envelope glycolipids and tetra-acylated sulfolipids, which are antagonists of TLR2, thereby inhibiting its role in pathogen recognition 21.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 1 September 2020 and 1 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 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 % ,

Exclusion criteria

1. All patients how have atopic diseases.
2. All patients how have autoimmune diseases .

Patients how have an infectious diseases

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.

PCR Master Mix volume :

Table (1) illustrate the volume for master mix of PCR

PCR mix		**Volum**	**Unit**
Master Mix (Bio-Lab.)		12.5	µL
Template of DNA		1.5	µL
Primer	Forward	0.5	µL
	Reverse	0.5	µL
Water free of nuclease		10.0	µL
Total		25.0	µL

TLRs Primers

Table (2) shown of TLRs primers sequences and product size.

Table (2)TLRs primers.

Gene	**Oligonucleotide Sequence (5'-3')**	**Amplicon Size, bp**	**Reference**
TLR2			
Forward	**CCAAGAGGAAGCCCAAGAAAG**	154	**Che,M. *et al.* 2017**
Reverse	**AAGTCCCGCTTGTGGAGACAC**		
TLR4			
Forward	**TTGAGCAGGTCTAGGGTGATTGAAC**	143	**Che,M. *et al.* 2017**
Reverse	**ATGCGGACACACACACTTTCAAAT**		
TLR9			
Forward	**AAGCTGGACCTCTACCACGA**	177	**Wujcicka, *et al.* 2015**
Reverse	**TTGGCTGTGGATGTTGTT**		

Thermal Cycler Programs Used in This Study

Table (3) illustrate the thermal cycles of PCR

Genes	**Temperature (˚C) / Time**					**Cycles No.**
	Initial denaturation	**Cycling conditions**			**Final extension**	
		denaturation	**annealing**	**extension**		
TLR2	94°C/30 sec.	94°C/30 sec.	59°C/60 sec.	68°C/1 min.	68°C/5 min.	30 Cyc
TLR4	94°C/30 sec.	94°C/30 sec.	65°C/60 sec.	68°C/1 min.	68°C/5 min.	30 Cyc

TLR9	94°C/30 sec.	94°C/30 sec.	59°C/60 sec.	68°C/1 min.	68°C/5 min.	30 Cyc

The Molecular DNA Extraction and Genotyping

DNA extraction

Blood DNA

The DNA extraction is illustrate in figure (1) was performed by using (Favogren Blood Genomic DNA Extraction Mini Kit), for purification of DNA from blood , according to the manufacturer's instructions as a follow:

1- Into a micro-centrifuge tube 200 µl of whole blood sample was shifted.

2- The sample was supplemented with 20 µl Proteinase K and 200 µl FABG Buffer. Pulse-vortex mix thoroughly.

3- The tubes had been incubated at a temperature of 60 oC for 15 minutes. The sample need to vortex during incubation every 3 to 5 minutes.

4- To avid slill drops inside of the lid the tubes have been spinned.

5- After addition 200 µl of ethanol (96- 100 %) to the sample must be mixed for 10 seconds thoroughly by pulse-vortex.

6- Spinning of the tubes inorder to remove all drops remained inside of the lid.

7- A FABG Mini Column was placed into a Collection Tube. The mixture (with any precipitate) carefully transfered to the FABG Mini Column, centrifuge at 6000 x g for 1 min and a new Collection Tube was placed to FABG Mini Column.

8- In the FABG Mini Column a 400 µl W1 buffer were added and a full speed centrifuge (18.000 x g) for 30 seconds the flow-through must discarded.

9- Into the FABG Mini Column a 750 µl Wash Buffer were added and for 30 seconds centrifuge at full speed and discard the flow-through again.

10- For 3 minutes at full speed centrifuge the column in order to dry it.

11- The FABG Mini Column was placed to a Elution Tube.

12- On the FABG Mini Column membrane center a 50 to 200 µl of Elution Buffer was added and for 3 minutes FABG Mini Column was standed.

13- To elute total DNA frequent centrifuge at full speed for 1 minutes.

14- Finally the total DNA was involved stored at 4 °C or -20 °C.

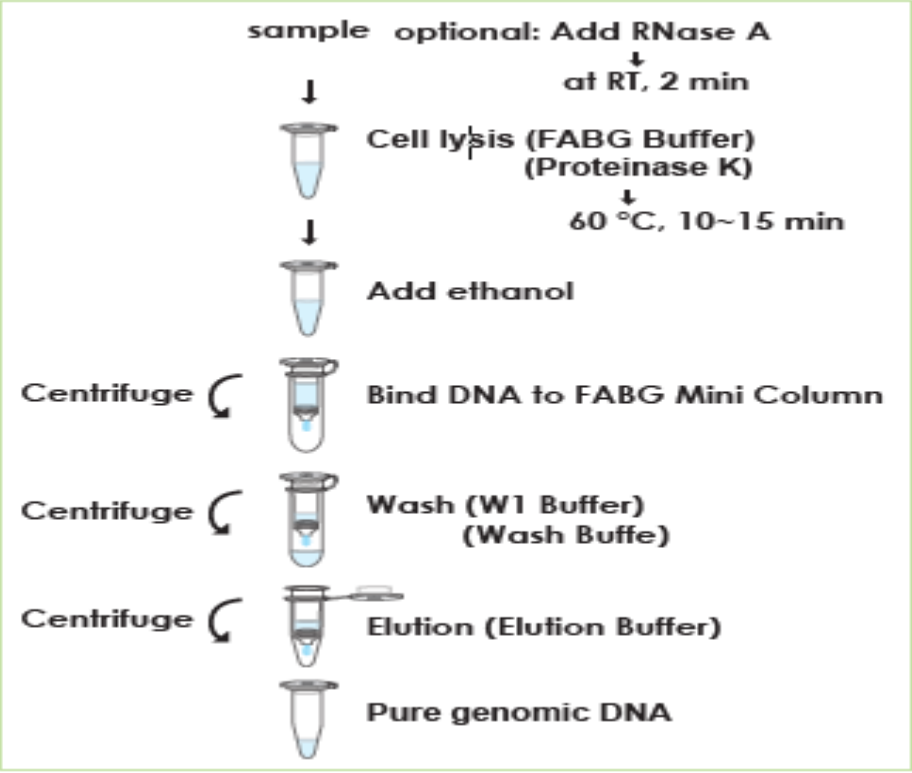

Figure (1) Blood DNA Extraction (LOT no.BHA23117A23, Favogren, Korea).

PCR amplification

The DNA of patients samples were tested with conventional PCR by using specific primer for TLR2,TLR4 and TLR9) to amplify an approximately sizes of each primers (Table 3). the pre reaction mixture was heated in a thermocycler for 30 seconds at 94°C and then submitted to 30 cycles of amplification with the three heating temperature of 94°C for 15-30 seconds , then with 59-65°C for 15-60 seconds , and 1 min at 68°C. The final extension step was done at 68°C for 5 min. according to manufactures instructions.

Detection of PCR results by agarose gel electrophoresis

The final PCR product was loaded on horizontal 1% agarose gels containing 0.2µg/ml ethidium bromide. The agarose gel preparation and running procedure was as follow:

1. After using 1X TBE in prepare of 1% Agarose gel, through dissolved in water bath at 100 °C for 15 minutes, left be colded to 50°C.
2. Added to the agarose gel solution 2 µL of ethidium bromide stain.
3. Followied fixing of the comb, the agarose gel solution was poured into the tray, left for 15 minutes at room temperature, then gently removes the comb and adding 10 µl of PCR product to each comb well, and 5 µl (1500 bp Ladder) to one well. Agarose gel solution was removed in a tray.

4. In the electrophoresis chamber, the gel plate was fixed and filled up with 1X TBE buffer. Electric current was then carried out for 30 minutes at 100 volts then 50 volt for 45 min.
5. PCR products were visualized by using ultraviolet trans illuminator.

DNA Sequencing

The sequence of the nucleotide of interleukins genes was known in 2samples, as 25 microliters of each sample of the PCR product with the Primers of the nucleotide of interleukin 4 gene were sent to Macro-gene in the Korea and after obtaining the results (Appendix 2) all the results were compared directly with the nucleotide of the nucleotide of interleukin 4 (IL4) Available in the internet (http: NCBI Reference Sequence) by computer program (BioEdit Pro. version: 7.0.0). The results were registered in NCBI under accession numbers (LC634417 & LC634418)Which is available on the website.

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, their age were rounded (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.

Molecular study

This section of study involved DNA extraction of whole blood from patients with tuberculosis and control group then followed by DNA amplification through using conventional PCR technique and specific primers.

DNA amplification

DNA extracted from whole blood samples have been amplified by using conventional PCR, then PCR product results confirmed by using 1% agarose gel electrophoresis (70 volts for 60min.), in this analysis the DNA band that appear on the gel after successful attachment between DNA template that had been extracted and the goal specialized primer for every one of choosed toll like receptors that have been studied (TLR2, TLR4 and TLR9), as seen in figure (2), the bands appeared under UV imaging system as orange compact bands due to the DNA staining that used as indicatorhich was ethidium bromide stain, the bands of extracted DNA can be estimated on gel electrophoresis by using DNA band size indicator that was (100-1500) DNA ladder, each Toll Like receptors (TLR2, TLR4 and TLR9) can be revealed on the results of amplified DNA, illustrated in figure (2) and table 4.

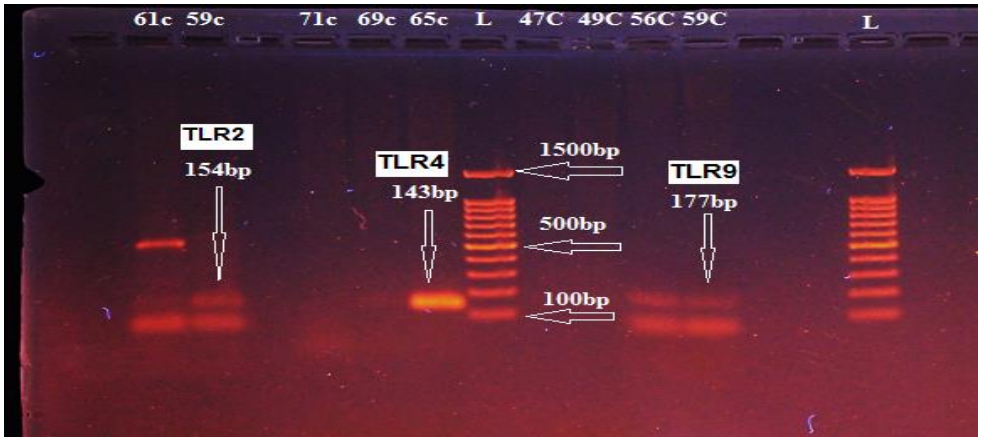

Figure (2): Shown PCR products for three primer sets (TLR2,TLR4 and TLR9) which shown 154bp, 143bp and 177bp respectively on (Agarose 1%, 70 volts 60min.).Visualized under UV imaging system after staining with Ethidium bromide, Lane L : DNA ladder (100-1500)bp

Table (4): Expression ratio of TLR2,TLR4 and TLR9 genes among TB patients.

No	Factors	No. of TB patients	No. of TB patients with positive results	Percent of positive results which gene expressed(%)
1	**TLR2**	88	75	85.2%
2	**TLR4**	88	88	100.0%
3	**TLR9**	88	87	98.8%

DNA sequencing

DNA sequencing for TLR2

TLR2 Forward primer identity

Toll like receptor 2 (TLR2) homo sapiens, a variant transcripted 8, the ID of mRNA. Sequence **:** NM_001318796.2 Extent: 3522 Matches Number: 1
, Range 1:2092 to 2204

Score	Expect	Identities	Gaps	Strand
182 bits(98)	3e-41	109/114(96%)	1/114(0%)	Plus/Plus

```
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Sbjct  2092  TGATGCAT-TTGTTTCTTACAGTGAGCGGGATGCCTACTGGGTGGAGAACCTTATGGTCC  2150

Query  77    AGGAGCTGGAGAACTTCAATCCCCCCTTCAAGTTGTGTCTCCACAAGCGGGACT  130
             ||||||||||||||||||||||||||||||||||||||||| || ||||||||||
Sbjct  2151  AGGAGCTGGAGAACTTCAATCCCCCCTTCAAGTTGTGTCTTCATAAGCGGGACT  2204
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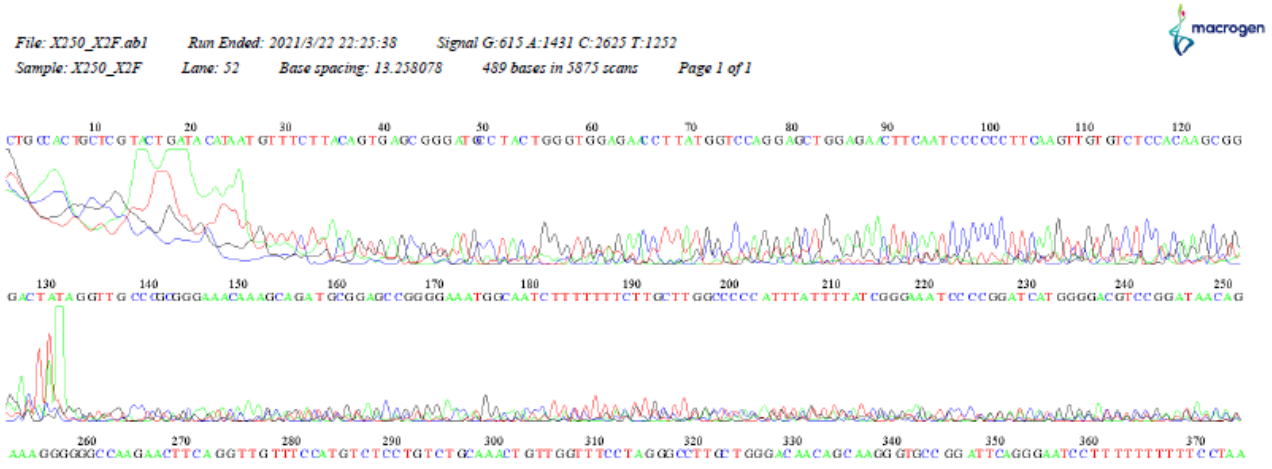
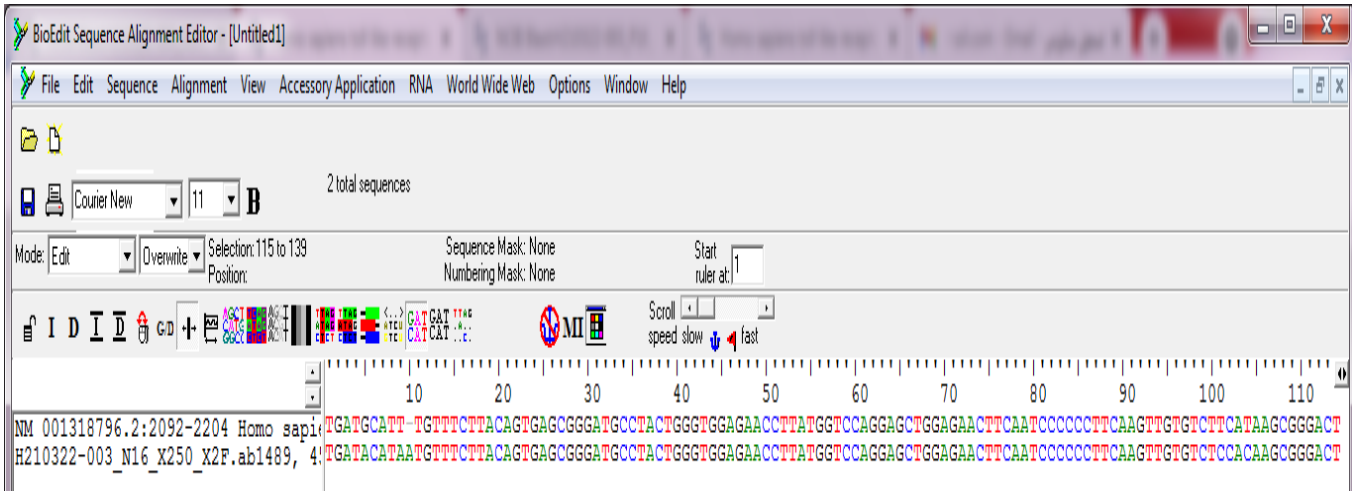


TLR2 Forward primer alignment and mutations observation

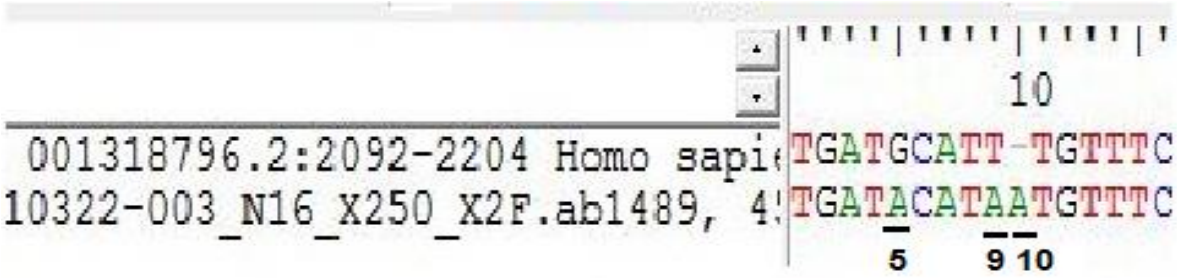
Table (5) and figure (3) were shown the most common types of mutations in the TLR2 forward gene, sequence in this study.

Table (5): Most common types of mutations in the TLR2 forward gene

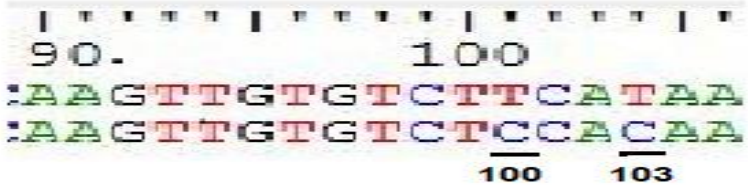
No. of sample F	Wild type	Mutant type	Site
50	G	A	5
50	T	A	9
50	-----	A	10
50	T	C	100
50	T	C	103



-A-



-B-



-C-

Figure (3): Most common types of mutations in the TLR2 forward gene (A,all mutations; B, Muations in sites 5-9-10 ; C, Mutations in sites 100&103).

Forward primer Phylogenetic tree

The TLR2 forward primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR2 sequences available in the Gen Bank database, there is no convergence between our TLR2 isolates and these of Gen Bank, as seen in the figure (4).

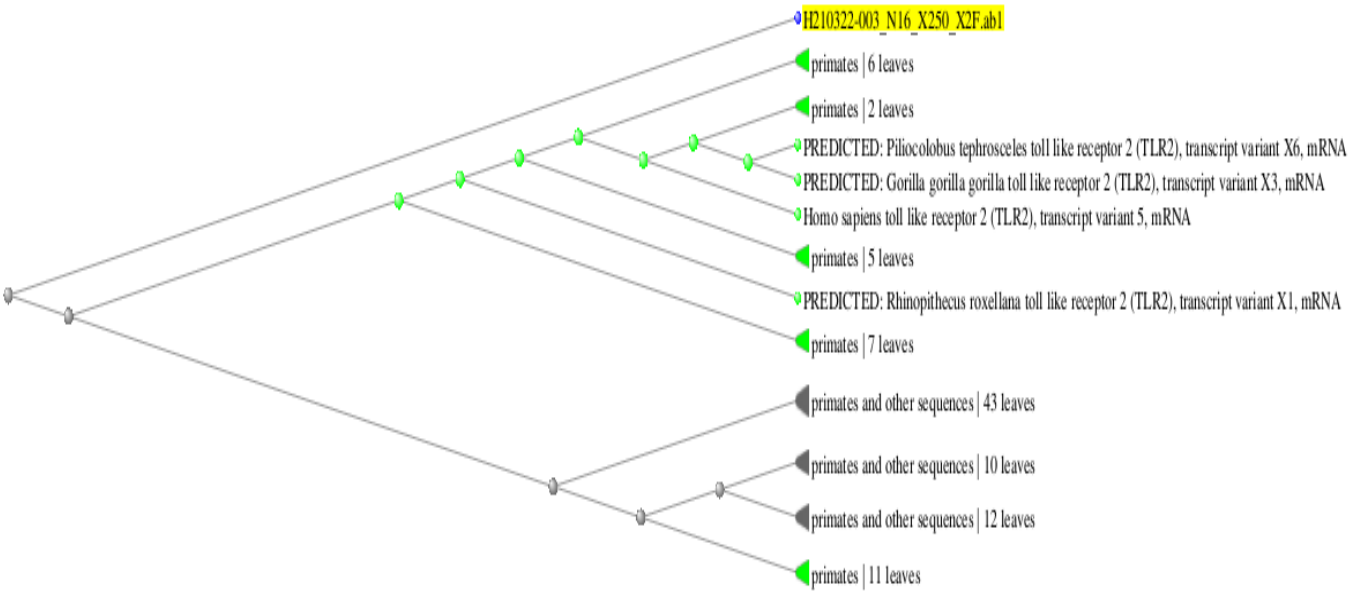


Figure (4): Shown TLR2 forward primer Phylogenetic tree analysis drow by Omega-7 program.

3.4.3.1.4 TLR2 Reverse primer identity

Toll like receptor 2 (TLR2) homo sapiens, a variant transcripted 8, the ID of mRNA. Sequence: NG_016229.1 Extent: 28803 Matches Number: 2
, Range 1: 25499-25606

Score	Expect	Identities	Gaps	Strand
189 bits(102)	4e-44	106/108(98%)	0/108(0%)	Plus/Minus

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Sbjct  25606  GCTCCTGGACCATAAGGTTCTCCACCCAGTAGGCATCCCGCTCACTGTAAGAAACAAATG  25547

Query  87     CATCATAGCAGATGTTCCTGCTGGGAGCTTTCTTGGGCTTCCTCTTGG  134
              |||||||||||||||||||||||||||||| |||||||||| ||||
Sbjct  25546  CATCATAGCAGATGTTCCTGCTGGGAGCTTTCCTGGGCTTCCTTTTGG  25499
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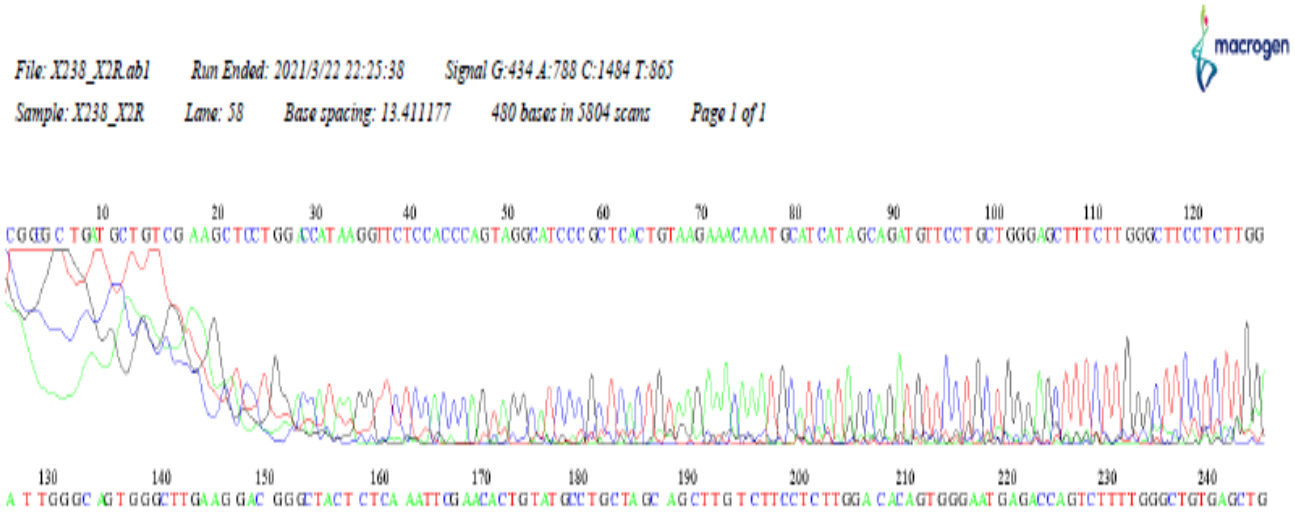

TLR2 Reverse primer alignment and mutations observation

Table (6) and figure (5) were shown the most common types of mutations in the TLR2 reverse gene, sequence in this study.

Table (6): Most common types of mutations in the TLR2 reverse gene

No. of sample F	Wild type	Mutant type	Site
50	T	C	119
50	C	T	130

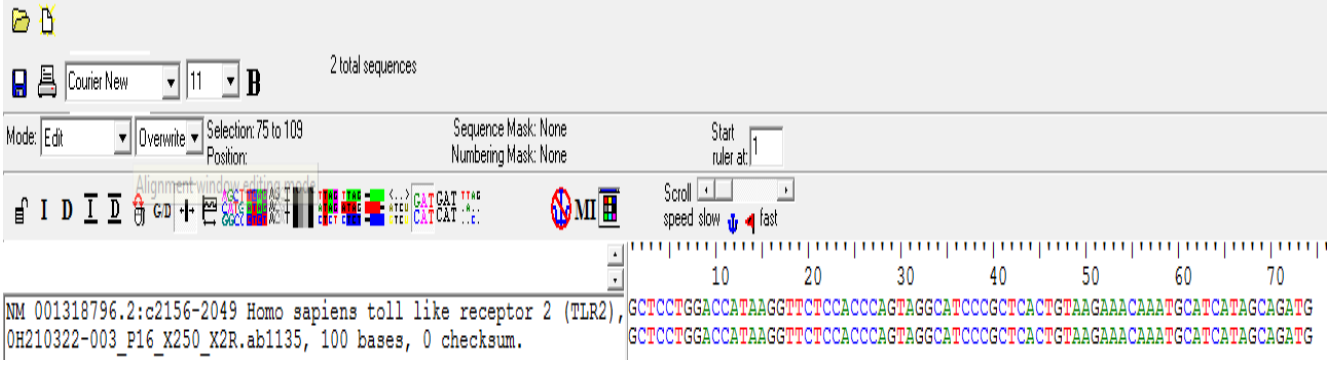

Figure (5): **Most common types of mutations in the TLR2 reverse gene.**

TLR2 Reverse Phylogenetic tree

The TLR2 reverse primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR2 sequences available in the Gen Bank

database, there is clearly convergence between our TLR2 isolates and these of Gen Bank.as seen in the figure (6).

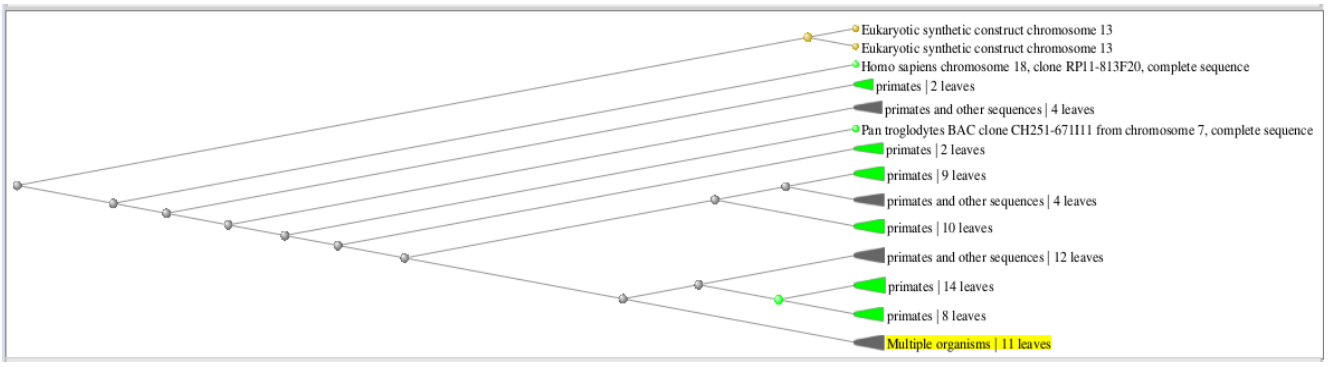

Figure (6) : Show TLR2 reverse primer Phylogenetic tree analysis drow by Omega-7 program.

DNA sequencing for TLR4

TLR4 Forward primer identity

Homo sapiens toll like receptor 4 (TLR4), RefSeqGene (LRG_320) on chromosome 9 Sequence ID: NG_011475.2 Length: 27333 Number of Matches: 1 , Range 1: 17613-17725

Score	Expect	Identities	Gaps	Strand
196 bits(106)	2e-46	111/113(98%)	1/113(0%)	Plus/Plus

```
Query  4      CTGGATGTGTTT-CATGTCTCATGTACTAGTGAAAGTAGATGTGTGCATTTGTGCACATA  62
              |||| |||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  17613  CTGGGTGTGTTTCCATGTCTCATGTACTAGTGAAAGTAGATGTGTGCATTTGTGCACATA  17672

Query  63     TCCCTATGTATCCCTATCAGGGCTGTGTGTATTTGAAAGTGTGTGTGTCCGCA  115
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  17673  TCCCTATGTATCCCTATCAGGGCTGTGTGTATTTGAAAGTGTGTGTGTCCGCA  17725
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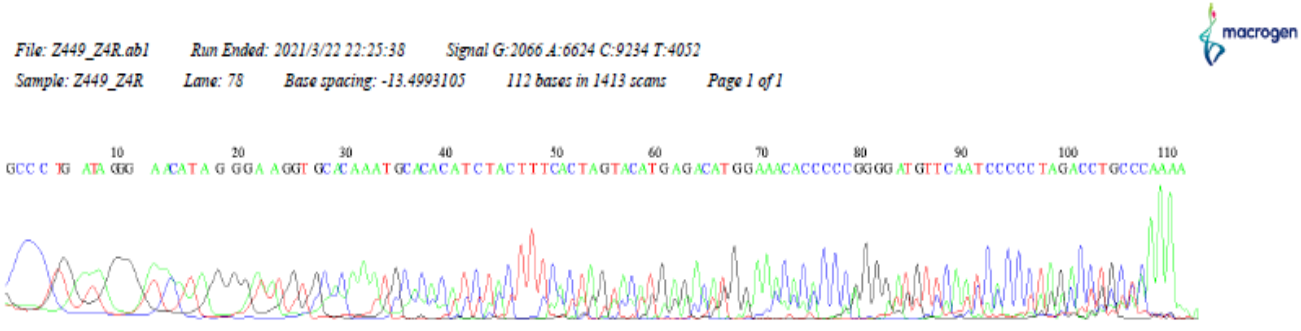


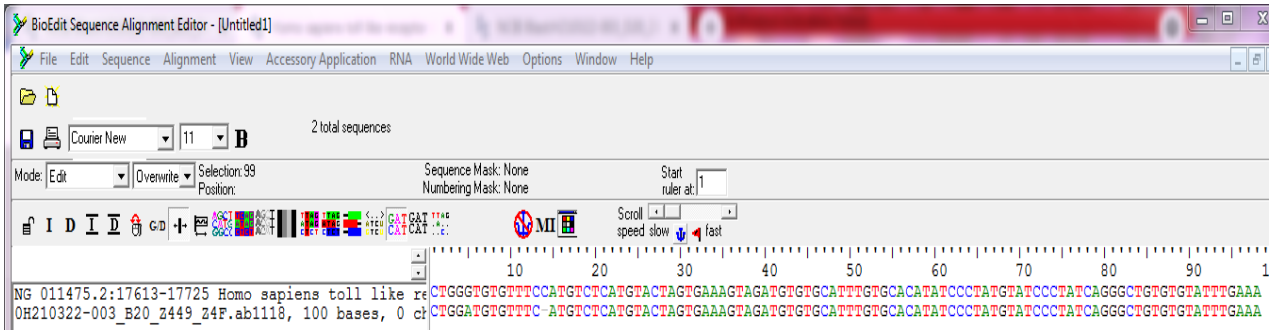
TLR4 Forward primer alignment and mutations observation

Table (7) and figure (7) were shown the most common types of

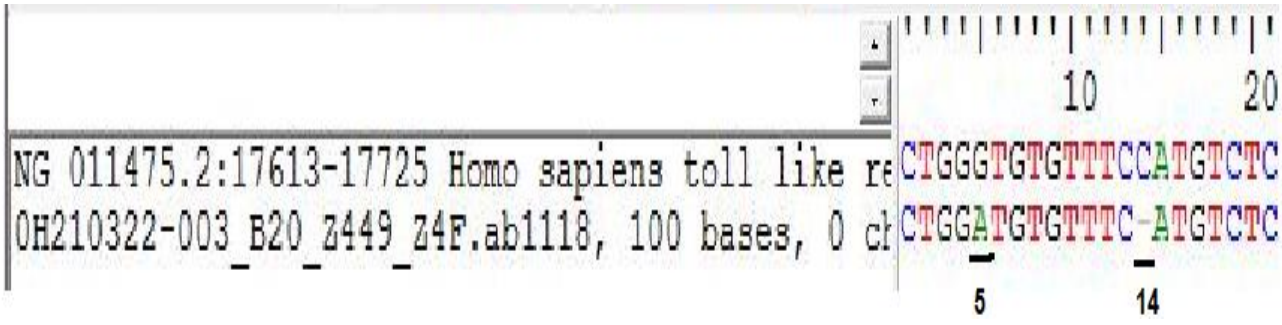
mutations in the TLR4 forward gene, sequence in this study.

Table (7): Most common types of mutations in the TLR4 forward gene

No. of sample F	Wild type	Mutant type	Site
49	G	A	5
49	C	-------	14



-A-



-B-

Figure (7) Most common types of mutations in the TLR4 forward gene (A,all mutations; B, Muations in sites 5-14).

TLR4 Forward Phylogenetic tree

The TLR4 forward primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR4 sequences available in the Gen Bank database, there is slightly convergence between our TLR4 isolates and these of Gen Bank.as seen in the figure (8).

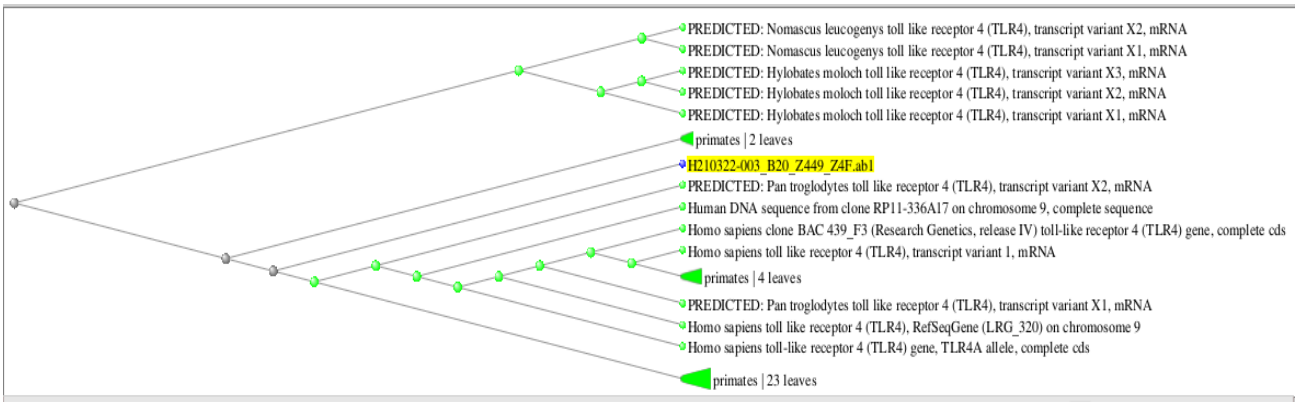


Figure (8) : Show TLR4 forward primer Phylogenetic tree analysis drow by Omega-7 program.

TLR4 Reverse primer identity

Homo sapiens toll like receptor 4 (TLR4), RefSeqGene (LRG_320) on chromosome 9 Sequence ID: NG_011475.2 Length: 27333 Number of Matches: 1 , Range 1: 17583-17695

Score	Expect	Identities	Gaps	Strand
169 bits(91)	4e-38	106/113(94%)	2/113(1%)	Plus/Minus

```
Query  1      GCCCTGATAGGGA-ACATAGGGA-AGGTGCACAAATGCACACATCTACTTTCACTAGTAC  58
              ||||||||||||| ||||||||| | ||||||||||||||||||||||||||||||||||
Sbjct  17695  GCCCTGATAGGGATACATAGGGATATGTGCACAAATGCACACATCTACTTTCACTAGTAC  17636

Query  59     ATGAGACATGGAAACACCCCCGGGGATGTTCAATCCCCCTAGACCTGCCCAAA  111
              ||||||||||||||||| ||| |||||||||||||| |||||||||||| ||||
Sbjct  17635  ATGAGACATGGAAACACACCCAGGGATGTTCAATCACCCTAGACCTGCTCAAA  17583
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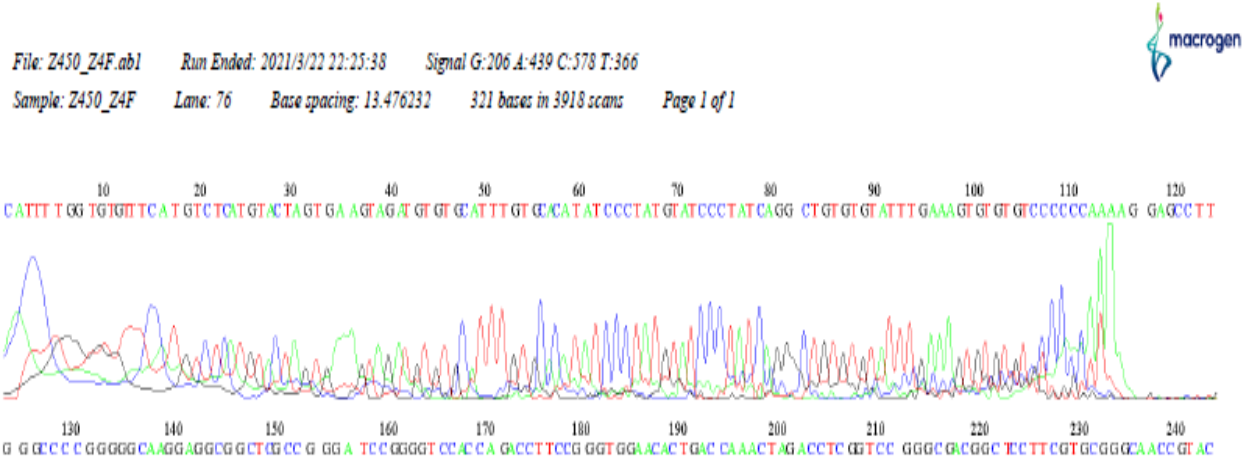
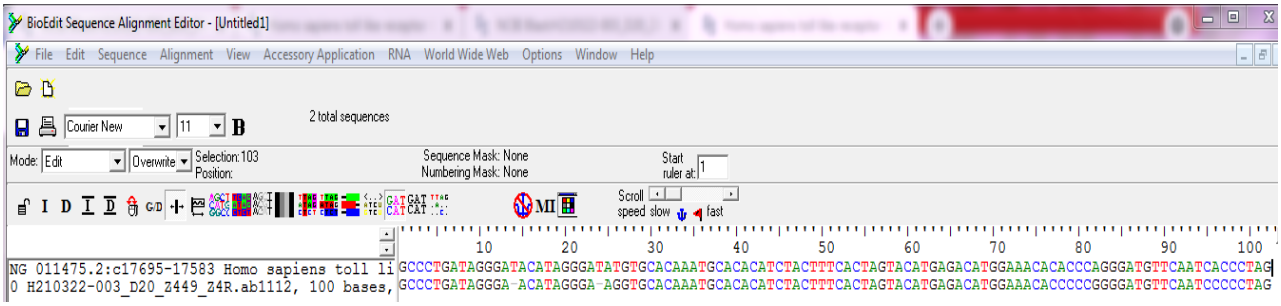


TLR4 Reverse primer alignment and mutations observation

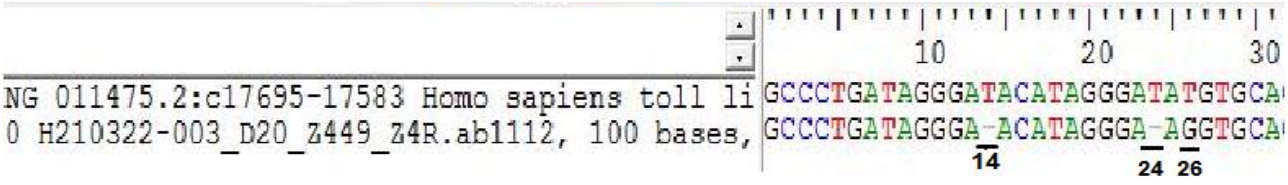
Table (8) and figure (9) were shown the most common types of mutations in the TLR4 reverse gene, sequence in this study.

Table (8): Most common types of mutations in the TLR4 reverse gene

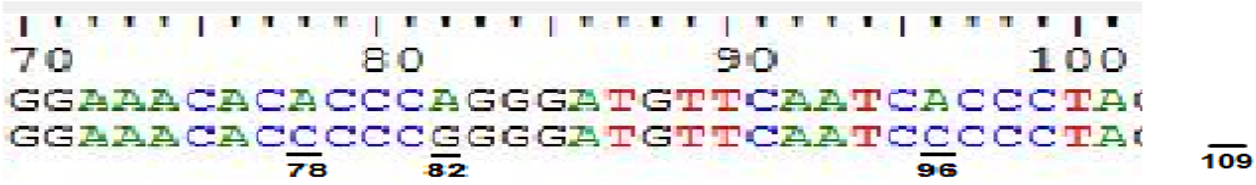
No. of sample R	Wild type	Mutant type	Site
49	T	-----	14
49	T	-----	24
49	T	G	26
49	A	C	78
49	A	G	82
49	A	C	96
49	T	C	109



-A-



-B-



-C-

Figure (9): **Most common types of mutations in the TLR4 reverse gene (A,all mutations; B, Muations in sites 14,24&26 ; C, Mutations in sites 78,82,96&109).**

TLR4 Reverse Phylogenetic tree

The TLR reverse primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR4 sequences available in the Gen Bank database, there is no convergence between our TLR4 isolates and these of Gen Bank.as seen in the figure (10).

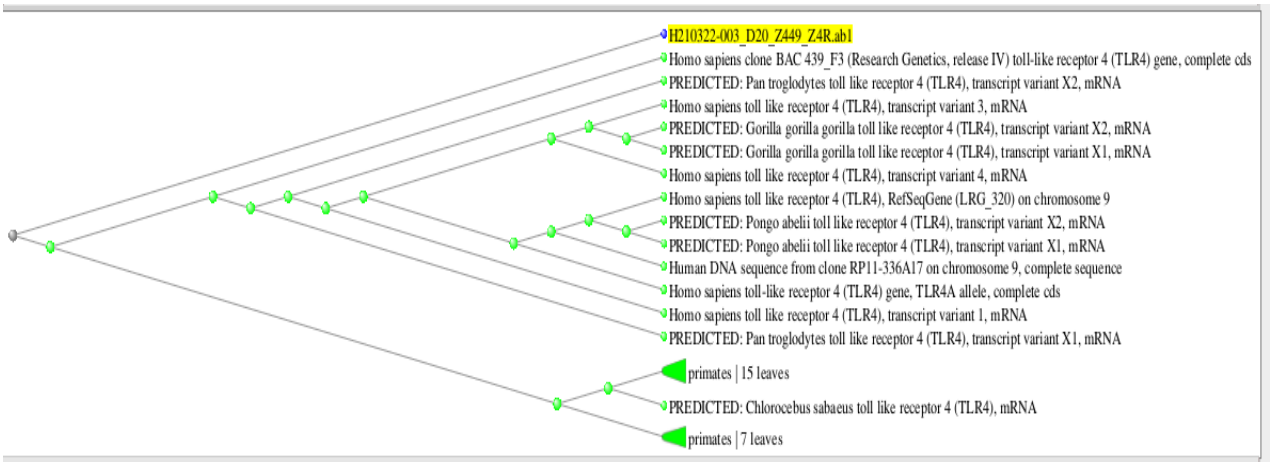


Figure (10): Shown TLR4 reverse primer Phylogenetic tree analysis drow by Omega-7 program.

DNA sequencing for TLR9

TLR9 Forward primer identity

Homo sapiens toll like receptor 9 (TLR9), RefSeqGene (LRG_320) on chromosome 3 Sequence ID: NG_033933.1 Length: 12084 Number of Matches: 1 , Range 1: 8486-8618

Score	Expect	Identities	Gaps	Strand
235 bits(127)	6e-58	132/134(99%)	1/134(0%)	Plus/Plus

```
Query  18    ACTGGAGGCCCTGGACCTCAGCTACAACAGCCAGCCCTTTGGCCATGCAGGGCGTGGGCC  77
             ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  8486  ACTGGAGGCCCTGGACCTCAGCTACAACAGCCAGCCCTTTGG-CATGCAGGGCGTGGGCC  8544

Query  78    ACAACTTCAGCTTCGTGGCTCACCTGCGCACCCTGCCCCACCTCAGCCTGGCCCACAACA  137
             ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  8545  ACAACTTCAGCTTCGTGGCTCACCTGCGCACCCTGCGCCACCTCAGCCTGGCCCACAACA  8604

Query  138   ACATCCACAGCCAA  151
             ||||||||||||||
Sbjct  8605  ACATCCACAGCCAA  8618
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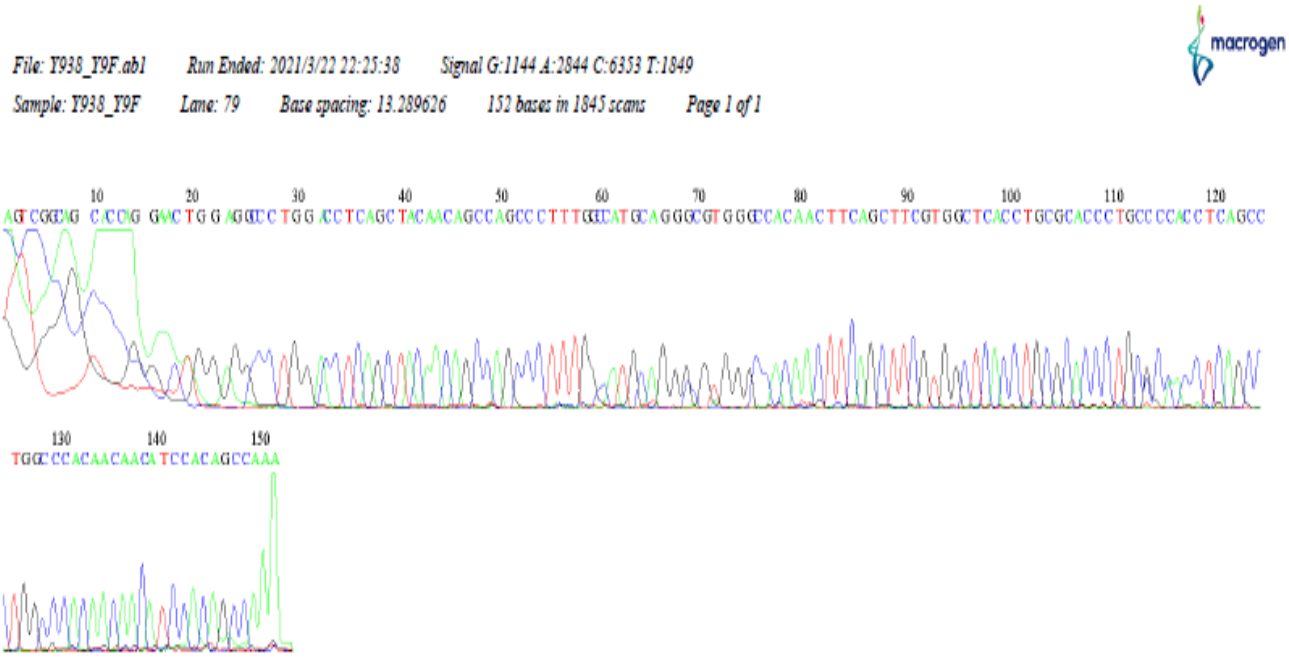
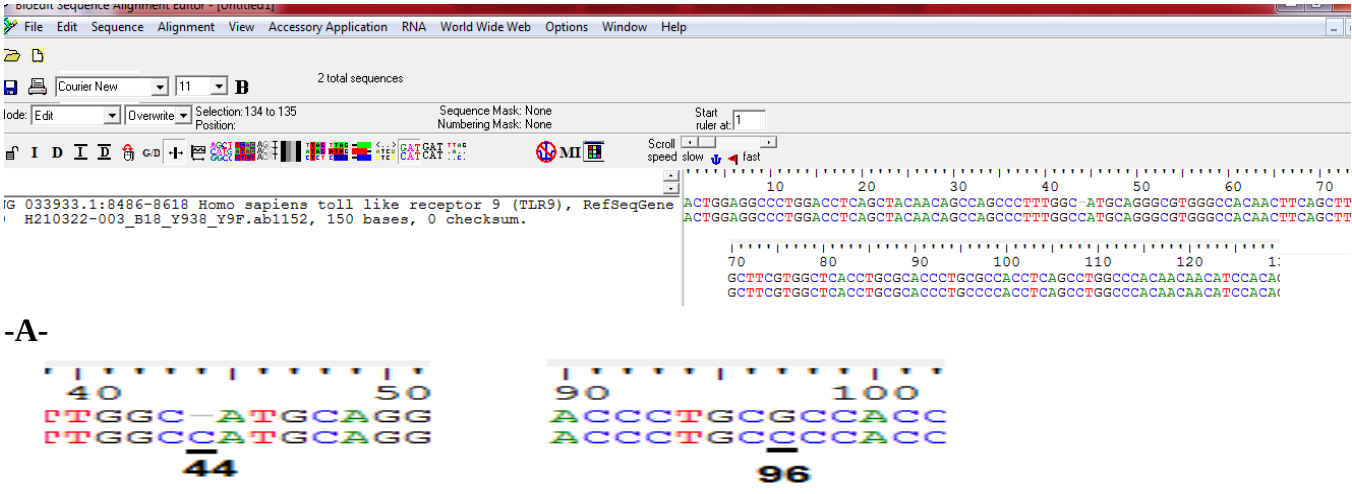


TLR9 Forward primer alignment and mutations observation

Table (9) and figure (11) were shown the most common types of mutations in the TLR9 forward gene, sequence in this study.

Table (9): Most common types of mutations in the TLR9 forward gene

No. of sample F	Wild type	Mutant type	Site
38	-----	C	44
38	G	C	96



-A-

-B-

Figure (11): Most common types of mutations in the TLR9 forword gene (A,all mutations; B, Muations in sites 44-96).

TLR9 Forward Phylogenetic tree

The TLR9 forward primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR9 sequences available in the Gen Bank database, there is no convergence between our TLR9 isolates and these of Gen Bank.as seen in the figure (12).

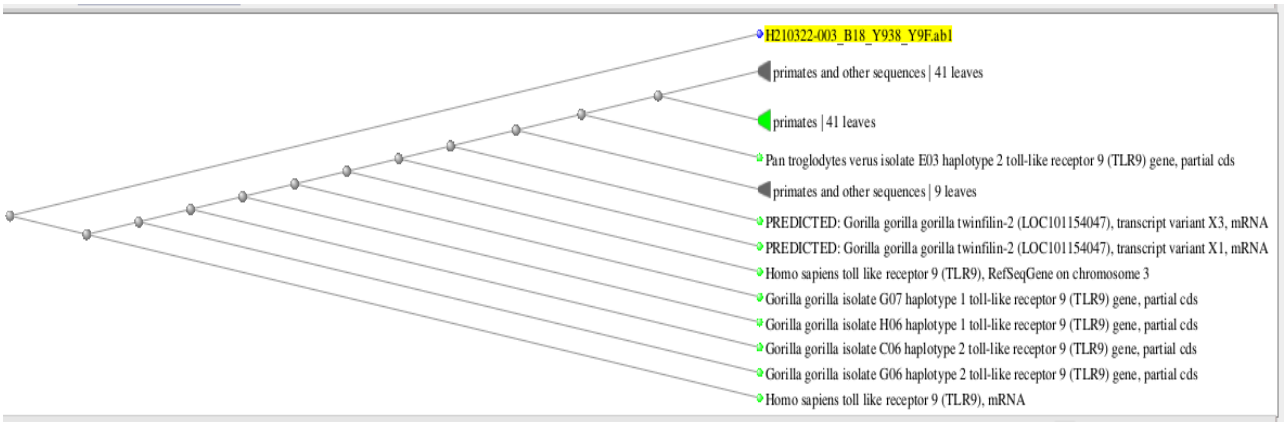


Figure (12): Show TLR9 forward primer Phylogenetic tree analysis drow by Omega-7 program.

TLR9 Reverse primer identity

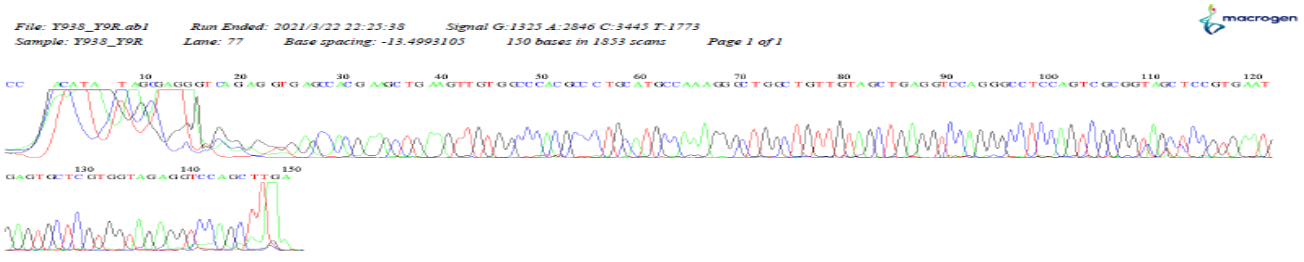
Homo sapiens toll like receptor 9 (TLR9), RefSeqGene (LRG_320) on chromosome 3 Sequence ID: NG_033933.1 Length: 12084 Number of Matches: 1 , Range 1: 8442-8569

Score	Expect	Identities	Gaps	Strand
237 bits(128)	2e-58	128/128(100%)	0/128(0%)	Plus/Minus

```
Query  21    AGGTGAGCCACGAAGCTGAAGTTGTGGCCCACGCCCTGCATGCCAAAGGGCTGGCTGTTG  80
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  8569  AGGTGAGCCACGAAGCTGAAGTTGTGGCCCACGCCCTGCATGCCAAAGGGCTGGCTGTTG  8510

Query  81    TAGCTGAGGTCCAGGGCCTCCAGTCGCGGTAGCTCCGTGAATGAGTGCTCGTGGTAGAGG  140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  8509  TAGCTGAGGTCCAGGGCCTCCAGTCGCGGTAGCTCCGTGAATGAGTGCTCGTGGTAGAGG  8450

Query  141   TCCAGCTT  148
              ||||||||
Sbjct  8449  TCCAGCTT  8442
```



TLR9 Reverse primer alignment and mutations observation

Figure (13) was shown no mutations in the TLR9 gene, sequence in this study.

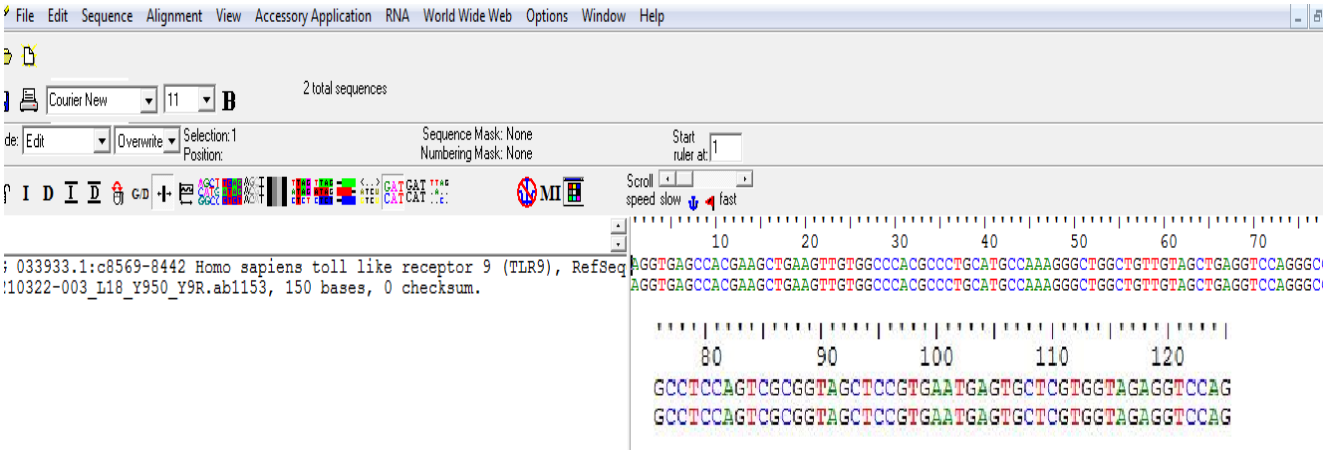


Figure (13): **No mutation in the TLR9 gene.**

TLR9 Reverse Phylogenetic tree

The TLR9 reverse primer isolate phylogenetic analyzes were analyzed by macrogen and compared with the different TLR9 sequences available in the Gen Bank database, there is highly convergence between our TLR9 isolates and these of Gen Bank.as seen in the figure (14).

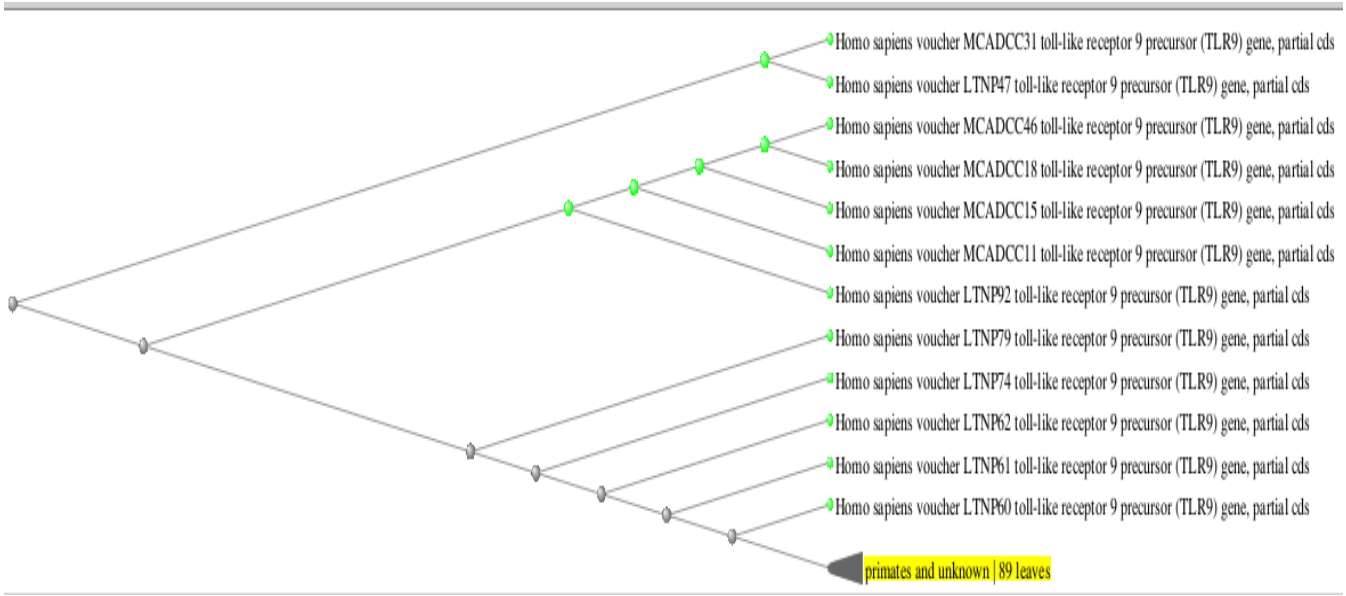


Figure (14): Show TLR9 reverse primer Phylogenetic tree analysis

A new recording genes for Toll Like Receptors

New toll like receptors gene was recorded in the Gen Bank after compared with different available genes in Gen Bank.

New recording gene of Toll Like Receptor2

Figure (15) was shown the nem recording gene of TLR2 in GenBank -**NCBI**

Figure (15): was shown the new recording gene of TLR2 in GenBank

New recording gene of Toll Like Receptor4

Figure (16) that shown the nem recording gene of TLR4 in GenBank

Figure (16): was shown the new recording gene of TLR4 in GenBank

New recording gene of Toll Like Receptor9

Figure (17) that shown the nem recording gene of TLR9 in GenBank

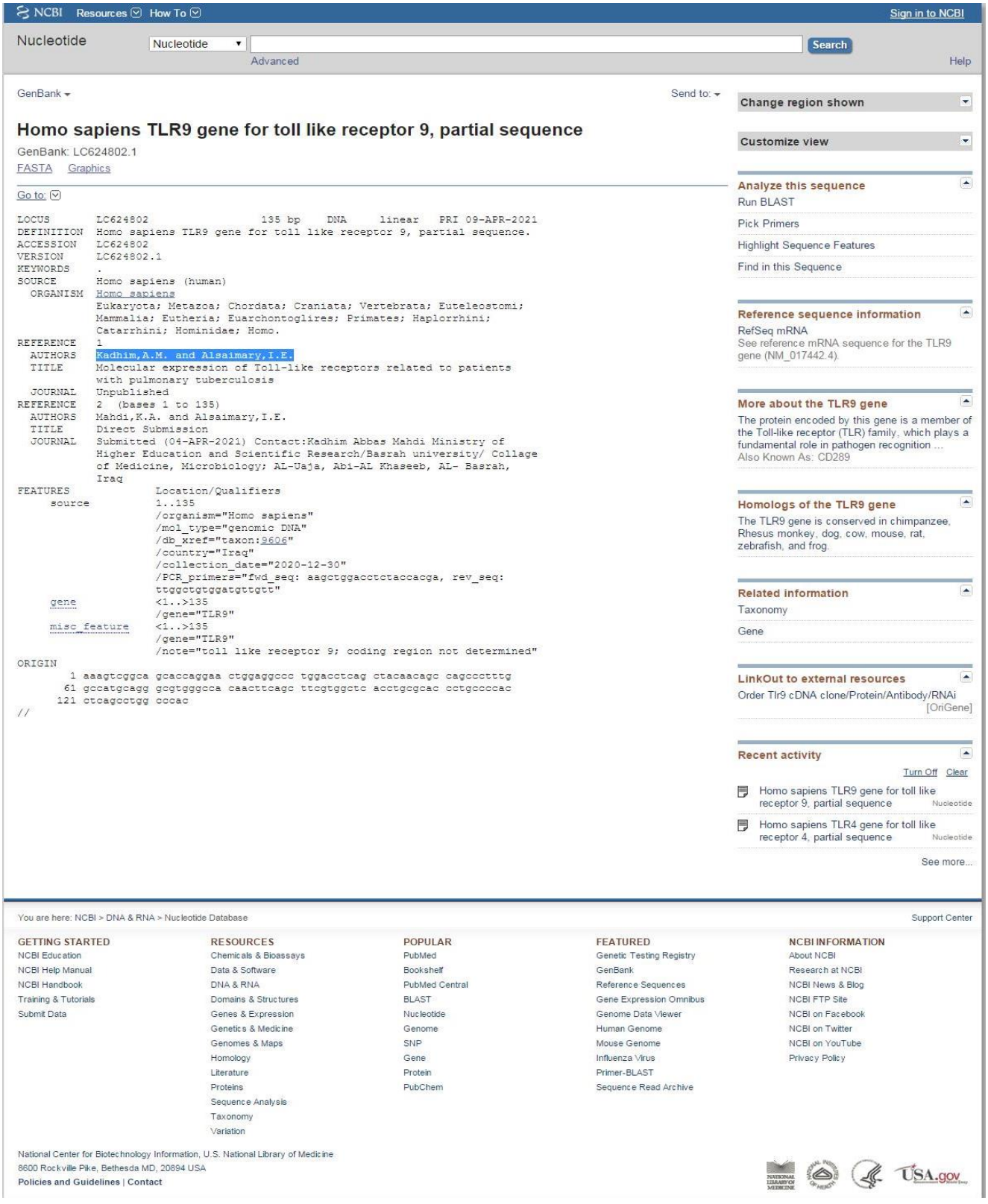


Figure (17): was shown the new recording gene of TLR9 in GenBank

The Discussion

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 third to fourth decades (30-39) were 16 (18.2 %) from total study patients, at the fourth to fifth decades (40-49) were 14 (15.9%), closely with it the first to secondth 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.

Toll Like Receptors (TLRs), a family of single membrane-spanning receptors, the nature involved thirteen types of TLRs, those 1 to 10 have been designated in humans, were expressed on cells of immune and non-immune system and others 11 to 13 in non-human beings **55** these innate immune sensors play critical roles at the interface of host mucosal immunity and TB 56. In the current study that selected of (TLR2, TLR4 and TLR9) to be study thier associasion with TB in Basrha province. Supported by other studies, TLRs had been recorded mainly in the recognition of tuberculosis were TLRs (2, 4, and 9) there was a possiblity TLR8 57. In addition study of 58 which selected these TLR genes (TLR2, TLR4 and TLR9), due to the strong biological evidence that supports their role in TB. In this results that the mean concentration of TLRs (ng/ml) was higher among tuberculosis patients 88(100.0), of TLR2 were (0.65 ±0.27), while in TLR4 (3.19 ± 1.78) (ng\ml), and TLR9 were (1.92 ± 1.06), statistically the differences was highly-significant.

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), 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 T-lymphocyte 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 was 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

TL9 a member of pattern recognition receptors (PRRs) plays critical role in host immune response against TB infection, and most frequently associated with the recognition of *Mycobacterium tuberculosis* PAMPs **68**. In this present study documented that the mean concentrations of TLR-9 (ng/ml) among male and female of tuberculosis patients (1.92±0.99) (1.95±0.99) respectively, was higher than male and female of control group (0.87±**0.**51) (0.82.±0.43) respectively, statistically the differences was highly significant with (P-value= 0.00,0.00) respectively. Other study evidenced as 2,3,9,11 which noted that TLR9 is known as the receptor for viral and

bacterial CpG-DNA, which also plays an important role in activating the innate immune system, shows the need for the linking of TLR9 to enable the immune response to the Th1. Supported by other study of 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 anti-tuberculosis 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 AL-BASRAH 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 Socio-economic 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
DOI Number: 10.5958/0974-1283.2019.00204.4
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 Socio-economic 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.