Interlukine-17 Polymorphism in Patients with Inflammatory Bowel Disease (IBD) in Al-Anbar Province

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Abstract

Tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) ARMS-PCR method was used to genotyping of IL-17A and IL-17F , The impact of IL-17A and IL-17F genotypes on the production of interleukins in IBD patients and control groups were determined and Interleukins gene polymorphisms role in IBD infection. significant deference at ($p \leq 0.05$) for each rs763780 in IL-17 F in patients especially Genotype TT,TC,CC were 31 (51.67%),19 (31.67%),10 (16.67%) \ and rs2275913 in IL-17 A in patients especially Genotype GG,GA,AA were 18 (30%),23 (38.33%),19 (31.67%). As well as genotyping of rs763780 in IL-17 F showed significant increase at a significant level of $P \leq 0.05$ in both TC and CC genotypes in the cases samples compared with the control samples. the odds ratio was 2.8894 and 22.3333 for TC and CC genotypes respectively at confidence interval up to 7.8 and 397 for both genotypes. T allele are more frequent in both cases and control and it have odds ratio as 5.021 And genotyping of rs2275913 in IL-17 A showed significant increase at a significant level of $P \leq 0.01$ for the AA genotyping in the cases samples compared with the control samples, while the GA genotyping did not show any significant differences,), the odds ratio for GA genotypes was 2.3590 at confidence interval up to 5.8, while it was 8.4444 at confidence interval up to 32.9 for AA genotypes. G allele are more frequent in both cases and control and it have odds ratio as 3.319 at confidence interval up to 6

Key words :*IL-17 polymorphism,IL-17, Inflammatory bowel disease.*

Introduction

Through the use of genome-wide affiliation scans, there had been 163 genetic chance loci that have been proven to make contributions to the chance of ulcerative colitis, Crohn's disease, or to both [(1).] Analyses of the genes and genetic loci implicated in IBD exhibit a number of pathways that are crucial for intestinal homeostasis, consisting of barrier function, microbial defense, epithelial restitution, innate immune regulation, reactive oxygen species, autophagy, law of adaptive immunity, endoplasmic reticulum stress, and metabolic pathways associated with mobile homeostasis [(2).] Numerous research have been pronounced the relationship between the polymorphism of IL-17A and IL-17F genes with specific inflammatory illnesses in extraordinary populations [(3,4).] Functional genetic polymorphisms in-17 and its receptor genes can affect both qualitatively or quantitatively their features [(5).] The genes for IL-17A and IL-17F are placed on chromosome 6p; the IL- 17B-encoding gene is positioned on chromosome 5q; that of IL-17C is on chromosome 16q, the IL-17D-encoding gene lies on chromosome 13q, and the gene for IL-17E was once mapped to chromosome 14q (6(.Moreover, quite a few research on single nucleotide polymorphisms have recommended that there is a connection between UC susceptibility and the IL-17 gene cluster [(7,8(.] Genetic polymorphisms in IL-17A and IL-17F may also have an effect on the expression of IL-17 by means of CXC chemokine induction and subsequent neutrophil recruitment, therefore affecting

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UC susceptibility [9] . A latest affirmation has proposed that genetic polymorphisms in IL-17A and IL-17F and serum IL-17 stages may also be concerned in the incidence of UC [10,11].

Materials and Methods

Patients and Controls :

The find out about blanketed (60)patients of unique a long time (15-60) who had been struggling from inflammatory bowel disease, who attended a prevalent educating medical institution in Ramadi metropolis in Al-Anbar governorate all through the length prolonged from the 1st of May 2020 to the 1st of February 2021. The samples of sufferers have been chosen According to the analysis of gastroenterologists. While manage covered (40) healthful folks of extraordinary a long time from (15-60) years. They had been viewed as a bad manage team as they did no longer exhibit a records of inflammatory bowel ailment after an investigation by way of gastroenterologists. Ten ml of venous blood was once accrued from a appropriate vein. Tourniquet was once utilized about (4-5) finger width above the chosen venipuncture website online and disinfected with 70% of Ethanol for 30 seconds and allowed to dry completely, the blood was once divided into two kinds of tubes, the first one; 2.5 ml complete blood was once distributed in in tow tubes with ethylene diamine tetraacetic acid tube (EDTA-tube) and blended gently, In the 2d tube; Residual phase of the blood pattern used to be transferred to it (free of anticoagulation) and let to coagulate for serum separation with the aid of the usage of a centrifuge at (4000 rpm) for 5 min, The remoted serum used to be accumulated in a sterile smooth white tube to be used for serological research Then tubes have been positioned in a cool-box underneath aseptic circumstance and saved in the freezer at (-20oC) till in addition processing [12,13].

Genetic Tests:

-Specific primer for human TLR4 Gene:

To detect the presence of Single Nucleotide Polymorphism (SNP) in the human *IL17F* and *IL17F* gene (rs763780 and rs2275913) primers were designed for tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) according to their sequence in NCBI https://www.ncbi.nlm.nih.gov pmc and by free online primer design tool http://primer1.soton.ac.uk/primer1.html, the primer sequence and product listed in the below table (1), which purchased from Macrogen company/Korea.

Table (1): Primers for detection of (rs763780 and rs2275913) in human TLR4 gene by Tetra-arms technique.

SNPs	Size (bp)	Annealing Temp.(°C)	Primer	Sequence
rs763780	C allele: 170	58.5	Inner F	/5- GGATATGCACCTCTTACTGCAAAC -/3
	T allele : 132		Inner R	/5- CTGTGAAGTGGAGGGAATTGG -/3
	Size of two outer primer :258		Outer F	/5- GTCACCCCTGTCATCCAACA -/3
			Outer R	/5- AAGGAAGACATCTCCATGAATTCC-/3
rs2275913	A allele:134	56	Inner F	5 TTCCCATTTTCCTTCAGACGG/3
			Inner R	/5-CCCCAATGAGGTCATAGAAGAATCTATT-/3
	G allele:194		Outer F	/5- TGACCCATAGCATAGCAGCTCTG-/3
	Size of two outer primer :279		Outer R	/5- GATGGATGAGTTTGTGCCTGCTAT-/3

Results and Discussion

Population Study:

The total number of persons included in the study was (100) samples which included (60) patients were found to have inflammatory bowel disease (cases) and (40) normal persons as control.

The Tetra ARMS-PCR Technique for rs763780:

The Tetra ARMS-PCR technique was used to genotype rs763780 in both cases and controls, The results of the statistical analysis showed a significant increase at a significant level of P≤0.05 in both TC and CC genotypes in the cases samples compared with the control samples where the frequency of TC genotyping was (31.67)% in cases samples, while the rate was (17.5)% in the control samples. For CC genotyping, the Frequency was (16.67)% in the cases samples, while the control samples did not show any frequency of CC genotyping. as show in table (2) and figures 1 and 2), the odds ratio was 2.8894 and 22.3333 for TC and CC genotypes respectively at confendence interval up to 7.8 and 397 for both genotypes. T allele are more frequent in both cases and control and it have odds ratio as 5.021

Table 2: Statistical evaluations of association between rs763780 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.

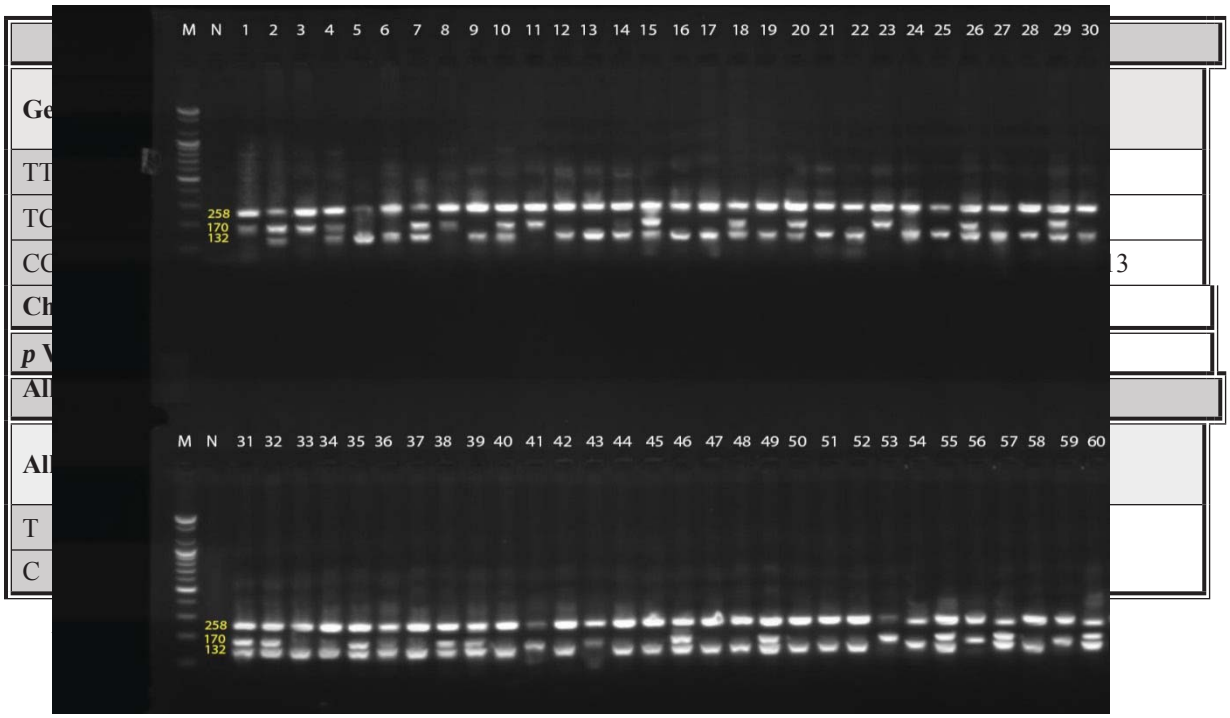

Figure (1) Tetra ARMS-PCR of *rs763780* gene polymorphism for cases samples

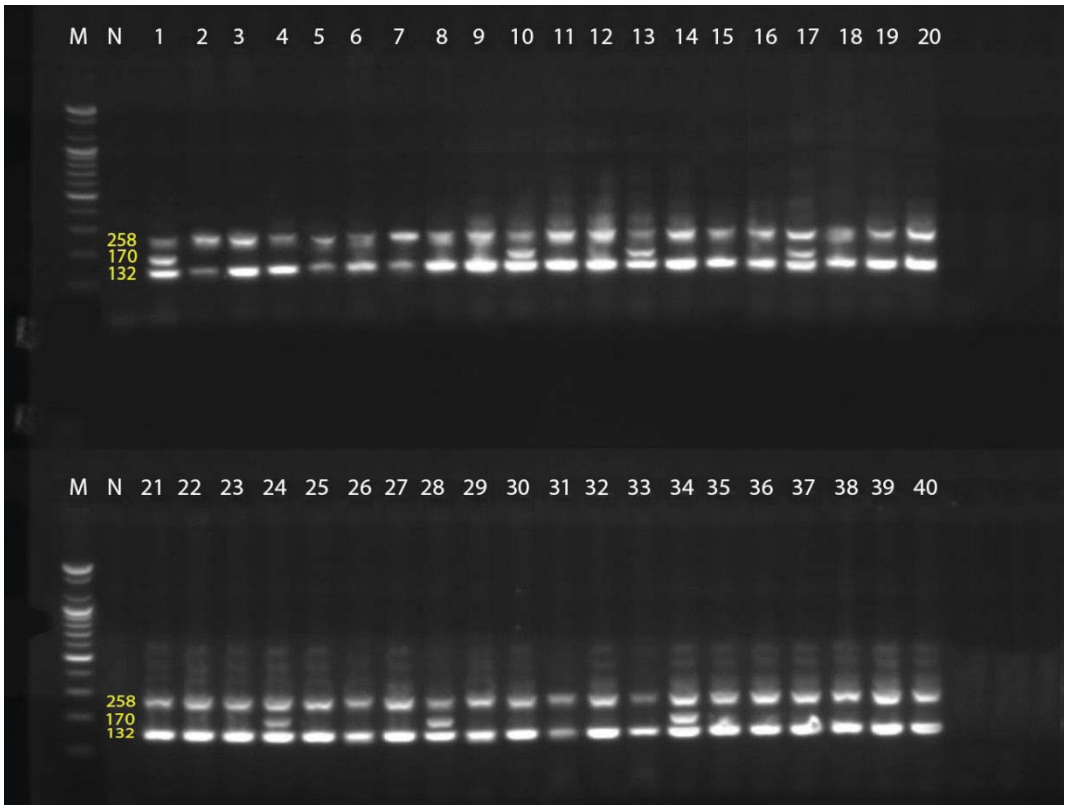

Figure (2) Tetra ARMS-PCR of *rs763780* gene polymorphism for controls samples

The Tetra ARMS-PCR Technique rs2275913:

The results of the statistical analysis of the genotyping of rs2275913 in both cases and controls showed a significant increase at a significant level of P≤0.01 for the AA genotyping in the cases samples compared with the control samples, where the frequency of genotyping AA was (31.67)% in the cases samples, while the frequency was (7.5). % In the control samples, while the GA genotyping did not show any significant differences. as show in table(3) and figures (3 and 4), the odds ratio for GA genotypes was 2.3590 at confendence interval up to 5.8, while it was 8.4444 at confendence interval up to 32.9 for AA genotypes. G allele are more frequent in both cases and control and it have odds ratio as 3.319 at confendence interval up to 6.0.

Table 3: Statistical evaluations of association between rs2275913 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.

rs2275913 Genotype Frequency (%)					
Genotype	**Control n=40**	**Patient n=60**	**p-value**	**Odds Ratio**	**95% CI**
GG	24 (60%)	18 (30%)	----	1.0	
GA	13 (32.5%)	23 (38.33%)	0.0658 NS	2.3590	0.9454 to 5.8860
AA	3 (7.5%)	19 (31.67%)	0.0021 **	8.4444	2.1621 to 32.9812
Chi-squared	0.4216 NS	3.261 NS			

Cont... **Table 3: Statistical evaluations of association between rs2275913 genotypes or allele in Inflammatory Bowel Diseases patients and control groups.**

P value	0.8099	0.1959			
Allele frequency (%)					
Allele	Control n=80	Cases n=120	p-value	Odds Ratio	95% Cl
G	0.95 (61)	0.58 (59)	0.0001**	3.319	1.776 to 6.096
A	0.05 (19)	0.42 (61)			

NS=Non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01

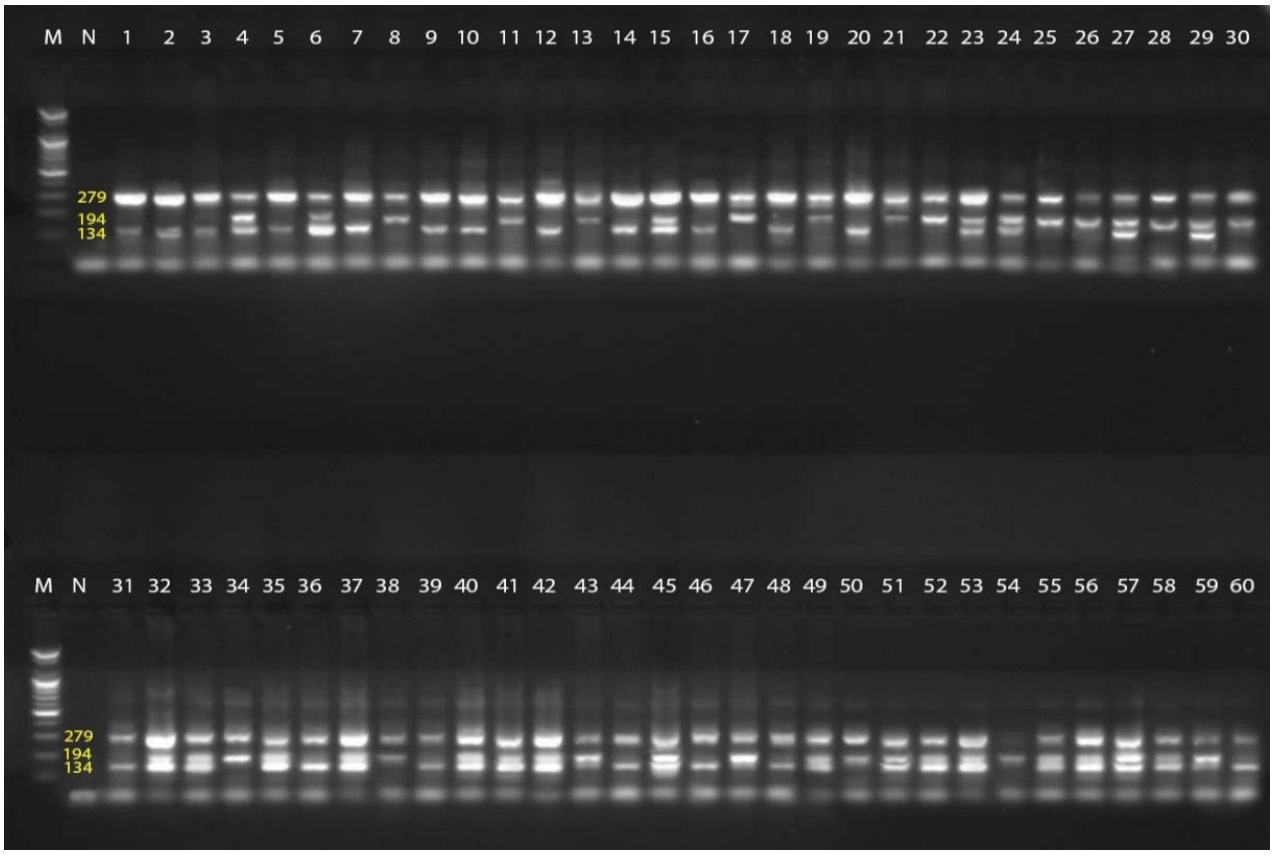

Figure (3) Tetra ARMS-PCR of *rs2275913* gene polymorphism for cases samples

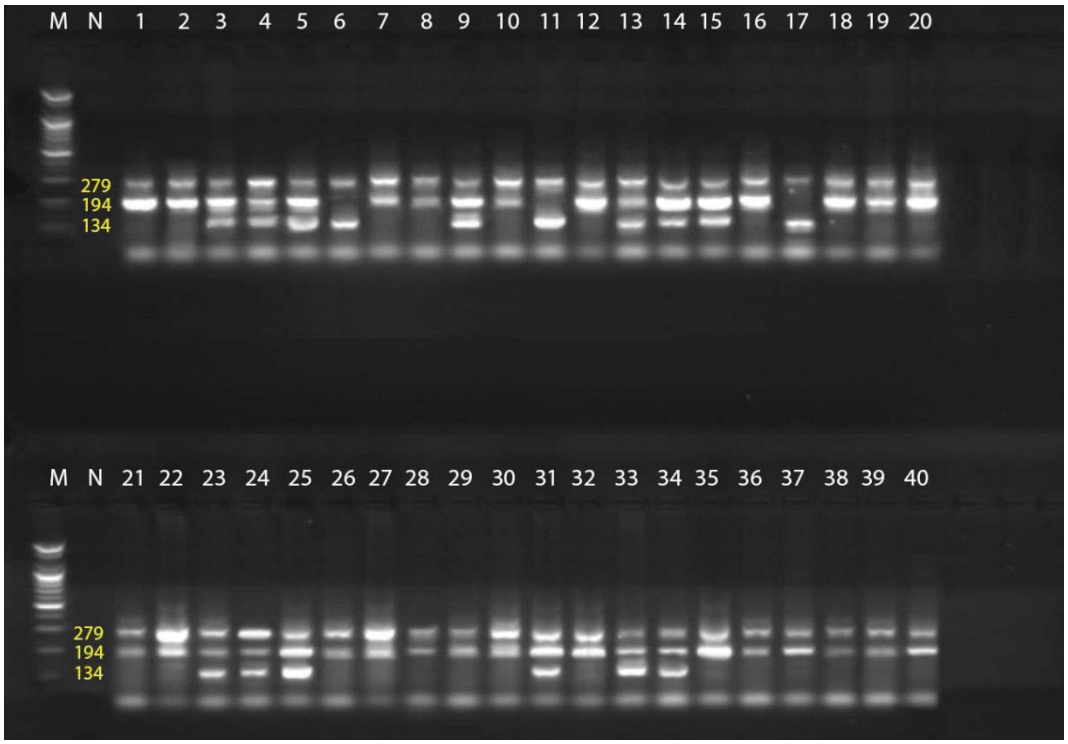

Figure (4) Tetra ARMS-PCR of *rs2275913* gene polymorphism for controls samples

The goal of our examination used to be to consider how rs2275913 and rs763780 genetic polymorphisms make a contribution to IBD and the relationship of IL-17A/17F genetic polymorphism with the scientific attributes. There is a restricted file that suggests the have an impact on of polymorphisms of IL17A and IL17F on the hazard of IBD, of all the SNPs, the IL17F (rs763780, 7488 T/C) polymorphism has been the most regularly stated As we exhibit in the end result of genetic polymorphisms for two SNPs the genotype TC and CC for rs763780 might also symbolize a threat issue for IBD whilst genotype AA may additionally be a danger genotype for rs2275913 and these consequences agree with many preceding outcomes posted to spotlight the impact of genetic polymorphisms of these SNPs in exclusive ailment and syndromes reviews confirmed a persisted make bigger in IL- 17-producing cells in the intestinal tissue of IBD patients, Over-expression of IL-17A or IL-17F in vivo leading to multiplied neutrophil infiltration thru modulation of cytokines and chemokines, main to infection [8]. The rs763780 SNP is placed in the role +7488 (coding region) of the IL17F gene, It consists of a substitution T to C, which leads to a trade in the amino acid sequence from Histidine to Arginine in function 161 (H161R) of IL17F protein, Reports mentions that the H161R variant of IL17F has no potential to set off the mitogen-activated protein kinase pathway, chemokine secretion in bronchial epithelial cells, and cytokine production, Furthermore, the H161R variant blocked the induction of the expression of IL8 by using wild-type IL17F, which performing as a herbal antagonist of the cytokine [14]. IL-17F rs763780 (His121Arg) polymorphism has been described to suppress the expression and the recreation of IL-17F and, this may additionally play a diverse function in the host's predisposition to inflammatory ailments [15]. A learn about shows that the rs763780 CC homozygous mutant genotype had an inverse correlation to ulcerative colitis susceptibility in the Chinese populace (p0.014), proposing that the rs763780 CC genotype would possibly be related with ulcerative colitis susceptibility; Another learn about referred to that the IL-17F 7488 TC polymorphism is considerably related with allergies (p0.0028) (16). A similarly sensible investigation in vitro suggests that the protein with rs763780 genotype can tighten the expression of wild-type rs763780 and discharge of the downstream cytokine [16,17,18]. Given that each allergies and ulcerative colitis have been believed

to be intervened obsessive immunoreactions by means of Th-2 cells, [19,20] the closeness in the relationship between IL-17F and the two awesome ailments seems to supply a signal to such a guiding principle and moreover recommends the doable cooperation between Th-17 and Th-2 cells in the pathogenesis of Inflammatory internal infection. [16] Nevertheless, the unique version principle suggests that few exceptional versions can also be aggregately answerable for a extensive extent of multifactorial inherited weak spot to IBD [21]. Further inspecting the connections between's the genotypes and scientific phenotypes of ulcerative colitis, it has been observed that the rs763780 TC heterozygote was once all the greater frequently related with the later establishing and mellow medical subtypes of the malady, demonstrating that IL-17F polymorphism might also emphatically affect disorder establishing and movement. [16] Arisawa et al.[22] determined that the IL17A (rs2275913, G-197A) and IL17F (rs763780, 7488T/C) alleles have been each essentially linked with an elevated chance of the development of ulcerative colitis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Detection of Capsular Polysaccharide Virulence Genes *rmpA* and *magA* of *Klebsiella pneumonia* Isolate from Diabetic Foot Ulcer Patient in Najaf Governorate in Iraq

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Abstract

Klebsiella Pneumoniae is an opportunistic pathogen, which mostly cause nosocomial infections. *K. Pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection. A total of (36) clinical swab specimens isolated from patient with diabetic foot ulcer in Al-Sdder Medical City (Central Diabetic Foot) in Najaf Governorate, Iraq from November 2019 to February 2020 , for detection some virulence capsular polysaccharide genes such as *rmpA* and *magA* that related with K1 serotype of *K . pneumonia* and associated with antibiotic resistance pattern in patient of diabetic foot ulcer. Antibiotic susceptibility test of *K. Pneumoniae* isolates against (20) of commonly used antibiotic showed there 25 (69%) of *K. pneumonia* isolate from diabetic foot patient were Multi-drug resistance (MDR),while11(31%) were extensive-drug resistance (XDR). The results of amplified product demonstrated that K1 serotype of *K . Pneumonia* was positive in 23 (64%) of totally clinical specimen than other types of serotype (Non-K1 serotype). *(magA*) gene that associated with hypermucoid viscosity was positive in 21 (58%) of total specimens while *(rmpA)* gene that regulate of capsular polysaccharide of *K .pneumonia* was positive in 22 (62%) of totally samples .

Keywords: *patients; Capsular polysaccharide; genes rmpA; and magA of Klebsiella pneumonia; toxicity*

Introduction

World Health Organization definition of the diabetic foot (DF) is pathologic consequences including infection, ulceration and or destruction of deep tissues associated with neurologic abnormalities, various degrees of peripheral vascular disease and/or metabolic complications of diabetes in the lower limb[(1)], Its long term complication that represent a major health problem of high mortality and morbidity rates. According to bacterial culture and molecular approaches, DFU can colonize with numerous aerobic and anaerobic polymicobial [(2)]. Among several types of bacteria that isolate from diabetic foot ulcer patient , *K .pneumonia* consider one of the most important causes in last year [(3)]. Virulence in *K. Pneumoniae* is based on many factors, but the capsule is regarded as the most important. The genomic study of these isolates mainly highlights capsular serotypes K1 and K2 and the presence of a large virulence plasmid which is responsible for hypermucoviscosity and which includes the mucoid phenotype gene (*rmpA*) regulator. Many virulence genes have been identified, some of which are associated with capsule polysaccharide production and a mucoid phenotype, such as *rmpA* and *rmpA2* . Hyper virulent and hypermucoviscous *Klepsiella* has emerged in the last few years. Hypermucoviscosity is a phenotype characterized by highly viscous and sticky colonies, and the term is often used in conjunction with hypervirulence[(4)]. Additional virulence factors in *Klebsiella* include:(i) adhesins (pili, fimbriae)(ii) siderophores, (iii) biofilm formation, and (iv) urease production. Nosocomial isolates of *K. Pneumoniae* often display multidrug-resistance phenotypes that are commonly caused by the presence of extended-spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for

treatment [5]. Capsule polysaccharide (CPS) have been classified into 80 serological types, termed K-antigens. The presence of the capsule is critical for the virulence of *K. Pneumoniae* [6]. Hypermucoviscosity has been related with the capsular serotype K1, and in a lower proportion with the serotype K2, and has been linked to the presence of the *magA* (mucoviscosisty-associated gene A) and *rmpA* (regulator of mucoid phenotype A) genes [7].The *rmpA* (regulator of mucoid phenotype A) gene is a plasmid-mediated confer a highly mucoviscous phenotype enhanced and regulator of the capsular polysaccharide synthesis. Nassif et al [7] explained that remove of the *rmpA* gene can decrease virulence in mouse lethality tests by 1000-fold. Deletion of *rmpA* result in reduction of cps synthesis that leads to decrease of bacterial virulence, in the other hand the strain contain mutation in this gene have defect on regular capsular polysaccharide biosynthesis this give the importance for hypermucoviscosity[8].The other gene associated with hypermucoviscosity carried by virulence strain is *magA* (mucoviscosity-associated gene A).It is a chromosomal gene, responsible for capsular polysaccharide synthesis K1, if mutation happened in *magA* lead to loss capsule[9]. The mechanisms resistance in *K. Pneumoniae* to various antibiotics classes included; production of antibiotic-inactivating enzymes, a variation of antibiotic target sites, changing of cell membrane permeability, efflux pump systems, and modification of metabolic pathways. High expression of β-lactamase as carbapenemases and cephalosporinases enzymes in *K. Pneumoniae* lead to increased resistant to β-lactam antibiotics. The cephalosporinases referred to as ESBLs [10]. Typically, *K. Pneumoniae* producing ESBL displayed resistance to β-lactam antibiotics like penicillin's, cephalosporin's, and monobactams. *K. Pneumoniae* carrying ESBL enzymes are frequently resistant to another class of antibiotics, including aminoglycosides, quinolones, and chloramphenicol. The spread of carbapenem-resistant *K.pneumoniae* isolates, both locally and internationally, creates a therapy problem due to less effective antibiotics for therapy, leading to an increase in the evolution of extensively drug-resistant (XDR) and pan drug-resistant (PDR) Gram-negative bacteria. Resistance to fluoroquinolones can be high; ciprofloxacin-resistant *K. Pneumoniae* has increased worldwide in recent years [11].

Materials and Methods

Specimens collection include, 36 specimens swab from diabetic foot infection ulcer from Al-Sadder medical city and outpatient in Najaf City between November 2019 to February 2020.The specimens collected were (21 Male and 15 Female) with age group between 39-70 years. A single colony was taken from each primary positive culture on blood agar, MacConkeys agar, mannitol salt agar and repeat growth for gain pure culture and then it was identified depending on its morphological and cultural characteristics. The hypermucoviscosity phenotype of the *K. Pneumoniae* isolates was determined using a modified string test. Isolates of *K. Pneumoniae* were cultured on MacConkeys agar and incubated for 24 hours at 37oC. The formation of a viscous string of at least < 5 mm was considered positive result[12].The Specimens collection that identified by using Vitec-2 automated system were 36, GN identification card have been used for identification Gram negative bacteria. All specimens of *K. pneumonia* that isolate in current study identify by using VITEC2- system, AST card use to detection antibiotic Susceptibility test [13]. Any bacterial strain that resist to a minimum of at least 3 different classes of antibiotics is of a multi-drug resistance (MDR), any bacterial strain that remains susceptible to only one or two classes of antibiotics is of extensive-drug resistance (XDR) and any bacterial isolate resistance to all sub classes in all classes of antibiotics is of a pan-drug resistance (PDR). Polymerase chain reaction assays were performed at a reaction volume of 50 µl. Depending on their reference procedure as in Table (1), to detection predominance of K1 serotype , *rmpA* and *magA* genes that associate with capsular polysaccharide virulence factor .

Table (1): Primers that use in this study

Name	Oligo sequence (5'-3')	Product Size	Reference
rmpA	**F**: ACGACTTTCAAGAGAAATGA **R** : CATAGATGTCATAATCACAC	409 bp	(15)
K1	**F**: GTAGGTATTGCAAGCCATGC R: GCCCAGGTTAATGAATCCGT	1045 bp	(18)
magA	F:GGTGCTCTTTACATCATTGC R:GCAATGGCCATTTGCGTTAG	1283 bp	(15)

Results

In this study, 19(53%) of total 36 *K.pneumoniae* that isolate from diabetic foot ulcer patient were positive while 17(47%) were negative . This result agreed with Aljanaby and Alhasani [14] which recorded (62.5%) of clinical isolated *K.pneumoniae* positive for hypermucoviscosity test and another Iraqi study in Baghdad show that (60%) of clinical isolate were positive [5].All specimens Identify and undergo antibiotic sensitivity test by using VITEC-2 system , The results show high resistance to Ampicillin, Amoxicalv and Ceftriaxone (100%) , followed by Cefazoline (94%) , Gentamicin (89%) and Ceftazidim (78%). *K. pneumonia* recorded low resistance to Impenem (33%) , Ertapenem (31%),Tigicycline (17%) and Amikacin (14%), where 25 (69%) were multi-drug resistance and 11 (31%) were extensive- drug resistance (XDR) , Table (2) :

Table (2) : The resistance profile of *K. pneumonia* to different antibiotics

No	Antibiotic Resistance Profile	Percentage	Type of Resistance
1	AX.AMC,CFZ,CEP,ERT,GNT,TOB, CRO,CIP,MOX,LEV,TIG,NIT,TRM	88%	XDR
2	AX,AMC,CFZ,CRO,CEP,AZT,ERT, CIP,MOX,TIG,NIT,TRM	75%	XDR
3	AX,CFZ,CRO,AZT,GNT,CIP,MOX,TRM	50%	MDR
4	AX,AMC,CFZ,CEP,AZT,IMP,MRP, AMK,GNT,MOX,CIP,NIT,TRM,TOB	88%	XDR
5	AX,AMC,CRO,AZT,AMK,GNT,TOB	44%	MDR
6	AX,AMC,CFZ,CRO,AZT,TRM	38%	MDR
7	AX,AMC,CFZ,CFX,CRO,CEF,CFZ, ERT,IMP, AMK,CIP,LEV,NIT	81%	XDR
8	AX,AMC,CFZ,CFX,CRO,CEF,CFZ ERT,IMP ,TOB,CIP,LEV,NIT	81%	XDR
9	AX,CFZ,CFT,CTZ,CRO,CEP,GNT,CIP, LEV,NIT,TRM	69%	MDR