Molecular assessment of Single-Nucleotide Polymorphisms (SNPs) of rs2910164G>Cgene that associated with Rheumatoid arthritis and Urinary Tract Infections

Hussein Khalid Zwain [1] Prof. Dr. Hawraa A.A.Al-Dahhan[2]

1. M.Sc. Hussein Khalid Zwain/ Pathological analysis Depart. / Faculty of Science / University of kuf

2. Prof. Dr. Hawraa A.A.Al-Dahhan/ Pathological analysis Depart./ Faculty of Science / University of kuf / hawraa.aldahan@uokufa.edu.iq

Abstract:

Recent study has demonstrated to detect the co-morbidity of UTI with RA by detecting the SNP of rs2910164G>Cgene and the most common genotype and allele frequency. This case-control study was performed on 145 patients with RA (70) and with UTI(75) and 80 healthy individuals. Tetra amplification refractory mutation system-polymerase chain reaction was used to genotype the rs2910164G<C gene polymorphisms by allele specific type. In RA patients the Genotypes (GC, CC) of rs2910164G>C gene polymorphism displayed a significant association (P≤0.05) in comparison with the control group and allele C rs2910164G>Cgene had a high ratio in RA (54.9 %) in comparison with the control group(45.1%). These results demonstrated that The rs2910164 G>C polymorphism was a risk factor for predisposition to RA. Furthermore, the rs2910164 G>C C allele was identified as a risk factor for susceptibility to RA (OR, 0.66; 95% CI, 0.417-1.057; P=0.084). In UTI patients genotypes of this gene (GG, GC, CC) rs2910164G>Cgene polymorphism displayed a non-significant association (P≤0.05) in comparison with the control group. allele G had a high ratio in UTI (77.33%) rs2910164 G>C gene was non-significant risk factor for predisposition of UTI patients to cause RA by recurrent infection .Furthermore, the rs2910164 G>C C allele was identified as non-risk factor for susceptibility to RA in UTI (OR, 1.595; 95% CI, 0.961-2.649; P=0.070) .

Key words: rs2910164G>Cgene , rs2910164G>Cgene , Single-Nucleotide Polymorphisms, UTI**.**

Introduction:

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory arthritis and extra-articular involvement. It is a chronic inflammatory disorder of unknown etiology that primarily involves synovial joints (Bartolomé-Casado et al.,2021).

Rheumatoid arthritis is a multifactorial disorder caused by a combination of environmental and genetic factors (Zhang et al.,2023). Family history; genetic and serological risk factors are more prevalent among first-degree relatives, hormonal factors, environmental antigens, nutritional factors, and exposure to infectious pathogens on mucosal surfaces such as urinary tract , all of these contribute to the development of Rheumatoid Arthritis (Cope, 2019).

Infectious agent play an important role in initiation of RA via a variety of mechanisms such as toll-like receptor, molecular mimicry or by direct invasion of synovial membrane(Kwon& Ju,2021). The clinical link between infection and RA has been established in several

investigations. Infection is frequently discovered early in the RA course and can occur before clinical arthritis, implying that infection plays a role in the onset and exacerbation of the disease (Li *et al*., 2013).

A urinary tract infection (UTI) is defined as an infection of the lower or the upper genitourinary tract that is diagnosed based on the presence of a pathogen in the urinary tract and the symptoms that accompany it (Al-Hamdani & Al-Hashimy,2020). Accumulating evidence points to *P. mirabilis* as an etiologic agent for rheumatoid arthritis in humans, which developed after UTI caused by this bacteria, broadening its pathogenic spectrum and value to public health (Rashid and Ebringer, 2007).Sera from rheumatoid patients exhibit higher levels of Proteus mirabilis IgM antibodies than those from other individuals (Abdul *et al*.,2023).

Genetic risk factors are considered to contribute at least two-thirds of the risk of RA (Yarwood *et al.,* 2015). Evidence from familial studies suggests that RA is caused by a combination of genetic and environmental factors. Large-scale genome-wide association studies (GWAS) have identified more than one hundred susceptibility loci for RA (Li *et al*.,2014 ; Mo *et al.*,2020). It has been reported that two single nucleotide polymorphisms (SNPs) rs2910164 in miRNA-146a and rs3746444 in miRNA-499 might be associated with susceptibility to RA (Li *et al*.,2014).

MicroRNAs (miRNAs) are short, non-coding epigenetic regulators that repress target gene expression mostly via binding to three′-untranslated region (3-UTR) of mRNA in a Dicer-dependent manner. Dysregulation of certain miRNAs can contribute to microglial hyper-activation, persistent neuroinflammation, and abnormal macrophage polarization in the brain. These abnormal conditions can support the pathogenesis of neurological disorders (Guo *et al*.,2019).

In rheumatoid arthritis (RA) many immune cells activate and induce a gene expression program to facilitate its replication and progression to disease. MicroRNAs (miRNAs) are key regulators of gene expression and could be involved during the infection. To address the genetic influence of miRNAs in RA and UTI, this study evaluated SNP of rs2910164 G.C(Chandan et al.2020).

The current study aims to detect the prevalence of) rs2910164 G<C SNP in RA patients in relation with UTI .In addition to the detect the most genotype of this SNP in RA &UTI patients and their associated with pathogenic bacteria isolates.

Materials and Methods

Patients:

This case control study was conducted to assess the polymorphisms of rs2910164 G/C gene in RA(70) and UTI(75) patients .Their age ranging between 25 to ≥ 60 that were attending the Rheumatology and UTI out patient's clinics ,respectively in Al-Sadder Medical City in Najaf/Iraq, during the period between November , 2022 to March, 2023. All RA patients diagnosed according to 2010 American college of Rheumatology/ European league Against Rheumatism (ACR/EULAR) . UTI patients diagnosed by physicians according to clinical symptoms and urine criteria(Welle ,2010). Eighty (80) age and gender matched healthy subjects taken as control. Patients with other inflammatory autoimmune disease were excluded from this study.

Samples Collection:

Five ml of the blood was obtained from all cases and controls . Two ml of the whole blood samples was transferred to EDTA tube For PCR (DNA extraction). The others 3 ml of the blood were used to obtain the serum after clotted for about 1 hr at room temperature. And centrifuged at 3000 rpm for 15 min, 0.5 ml of serum was separated plain tube for (Anti-CCP and RF Tests).

Genomic DNA extraction:

Genomic DNA was extracted from peripheral blood of all samples by a commercial nucleic acid extraction kit (BIONEER/ Korea) according to the manufacturer's instructions.

Detection of SNP(single neucleutid polymorphism) of rs2910164 gene:

Tetra amplification refractory mutation system-polymerase chain reaction method (T-ARMS-PCR) is a simple and rapid method with an important level of accuracy for the detection of genotyping of the rs2910164 G/C polymorphisms. Genotyping of rs2910164 was performed using two outer primers (FO and RO) and two inner allele-specific primers (FI and RI) for each SNP(Table I).

PCR was performed using commercially available PCR premix (AccuPower PCR PreMix; Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. The PCR cycling conditions were as in table (2).

The PCR products were verified as 1.5% agarose gels containing 0.5 µg/ml DS red stain and observed under UV light. The product sizes for rs2910164 were 170 bp for the C allele and 250 bp for the G allele, while the product size for the two outer primers (control band) was 360 bp (table. 1).

Table (1): The primers used for detection SNPs in rs2910164 G>C.

Gene type		Primer sequence (5'-3')	Product size(bp)	Reference
rs2910164 G>C	FO	GGCCTGGTCTCCTCCAG ATGTTTAT	360	Primer design by (NCBI)
rs2910164 G>C	RO	ATACCTTCAGAGCCTGA GACTCTGCC		
rs2910164 G>C	FI (C allele)	ATGGGTTGTGTCAGTGT CAGACGTC	170	
rs2910164 G>C	RI (G allele)	GATATCCCAGCTGAAGA ACTGAATTTGAC	250	

Tble (2): **PCR thermos cycling conditions** of rs2910164 G>C gene

Gene	Initial Denaturation	Cycles			Final elongation
		Denaturation	Annealing	Elongation	
rs2910164G>C (FO)	95°C /5 min.	35cycles			72˚C/5 min.
		95˚C/30 sec.	60°C/30 sec.	72°C/40 sec.	
rs2910164G>C (RO)	95°C /1min.	95°C/30 sec.	60°C/30 sec.	72°C/40 sec.	Seventy-two°C/5 min

rs2910164 G>C (FI) (C allele)	95°C/1 min.	95°C/30 sec.	60°C/30 sec.	72°C/40 sec.	Seventy-two°C/5 min
rs2910164 G>C (RI) (G allele)	95°C/1 min.	95°C/30 sec.	60°C/30 sec.	72°C/40 sec.	Seventy-two°C/5 min

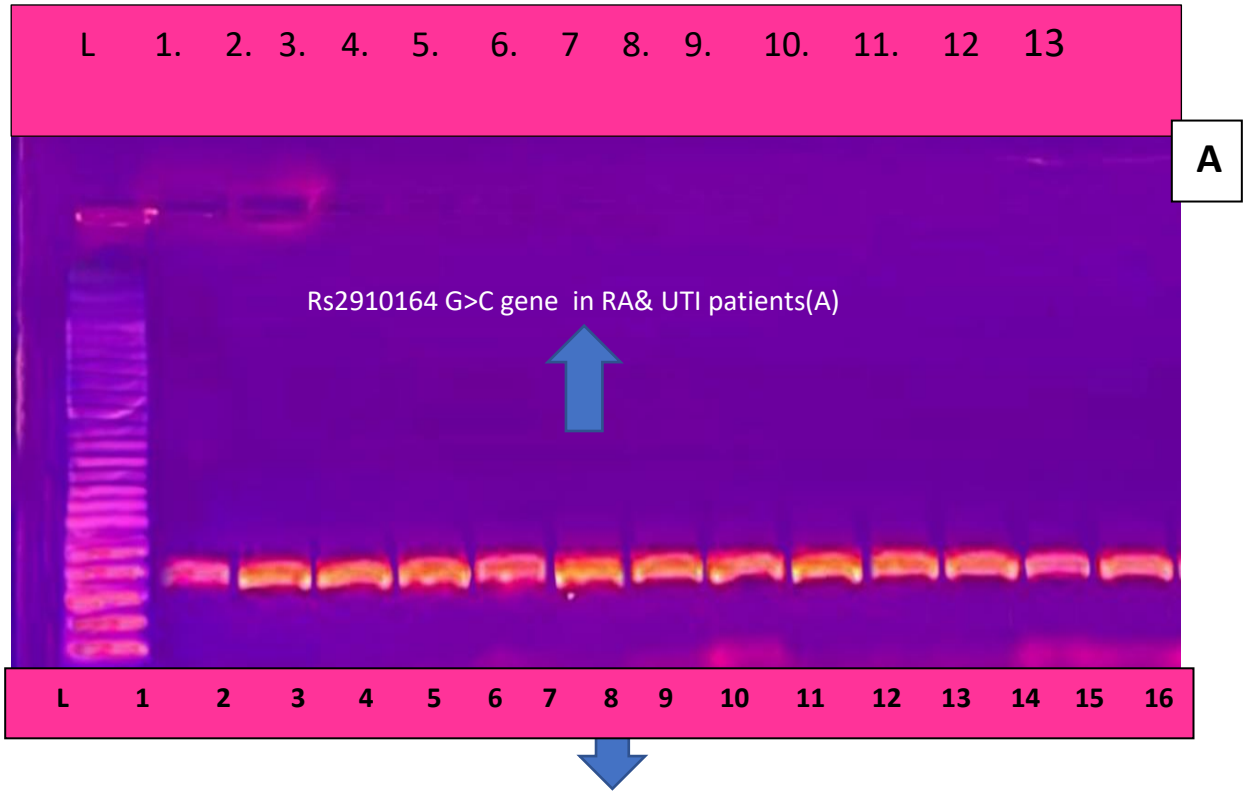
Statistical analysis.

Statistical analysis was calculated using SPSS 20 software. The genotype and allele distributions were determined in each group, and the odds ratios (OR) with 95% confidence intervals (95% CI) have been calculated. Also, a p-value>0.05 was considered statistically significant at a confidence interval (CI) of 95% .

Results and Discussion

The analysis of the association between rs2910164G>Cgene SNPs and the its present in RA and UTI patients was done by using Allele-Specific PCR methods.

Figure (1) show the presence of rs2910164G>C gene In patients and control groups.



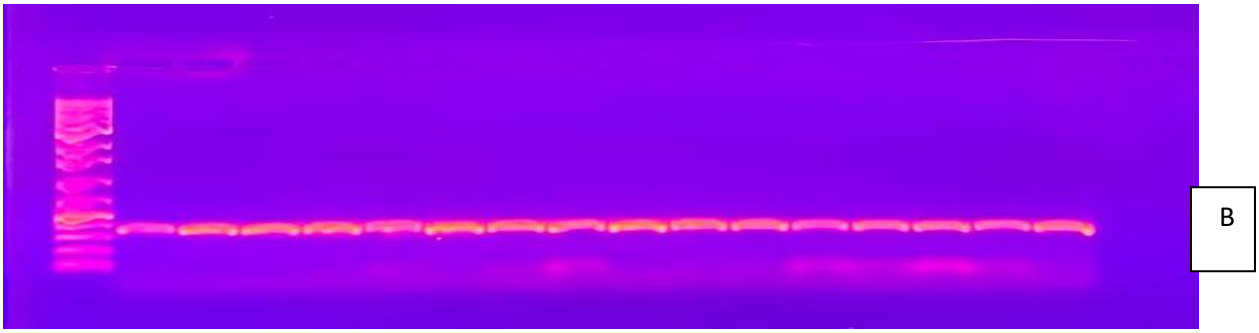


Figure (1) Agarose gel electrophoresis (1.5%, 1x TBE) showing PCR product of rs2910164 G>C gene (360bp) by using RO&FO as primers .(A) Lane 1-8 represented the gene product in RA patients, while the lane 9-13 showed the gene product in UTI patients.(B) represented the presence of rs2910164 G>C gene (360bp) in control . Agarose gel at 80% volts for one hour, Lane L: 100-1500 bp ladder

- Molecular Assessment of rs2910164G>Cgene Polymorphism that Associated with RA

In RA patients the genotype and allele frequency of rs2910164G>C gene polymorphism are shown in table (2) and figure (2). Genotypes of this gene (GC, CC) rs2910164G>C gene polymorphism displayed a significant association (P≤0.05) in comparison with the control group. While the Genotypes of rs2910164G>C gene (GG) showed no significant association between RA and the control group. In addition to that, allele C rs2910164G>Cgene had a high ratio in RA (54.9 %) in comparison with the control group(45.1%). In contrast, allele G rs2910164G>C gene had a high ratio in the control group (55.3 %) in comparison with the RA group (44.7%) as in table (2).

The frequency distribution of rs2910164 G>C genotypes in RA patients and normal control group are demonstrated that The hsa-mir- rs2910164 G>C polymorphism was a risk factor for predisposition to RA .Furthermore, the rs2910164 G>C C allele was identified as a risk factor for susceptibility to RA (OR, 0.66; 95% CI, 0.417-1.057; P=0.084)

Tble (4-5): Distribution of the genotypes (GG, GC, CC) and alleles (G, C) of the rs2910164 G>C gene in Rheumatoid arthritis patients.

Genotype/ allele	RA (No. =70)	Control(No.= 80)	P. value	OR	C.I 95%
Genotype					
GG	34	42	0.0844(NS)	0.6641	0.4172-1.0572
GC	10	25	0.0164(S)	0.3667	0.1616-0.8322
CC	26	13	0.0044(S)	3.0455	1.4147-6.5561
Allele					
G	88	109	0.084(NS)	0.66	0.417-1.057
C	62	51			

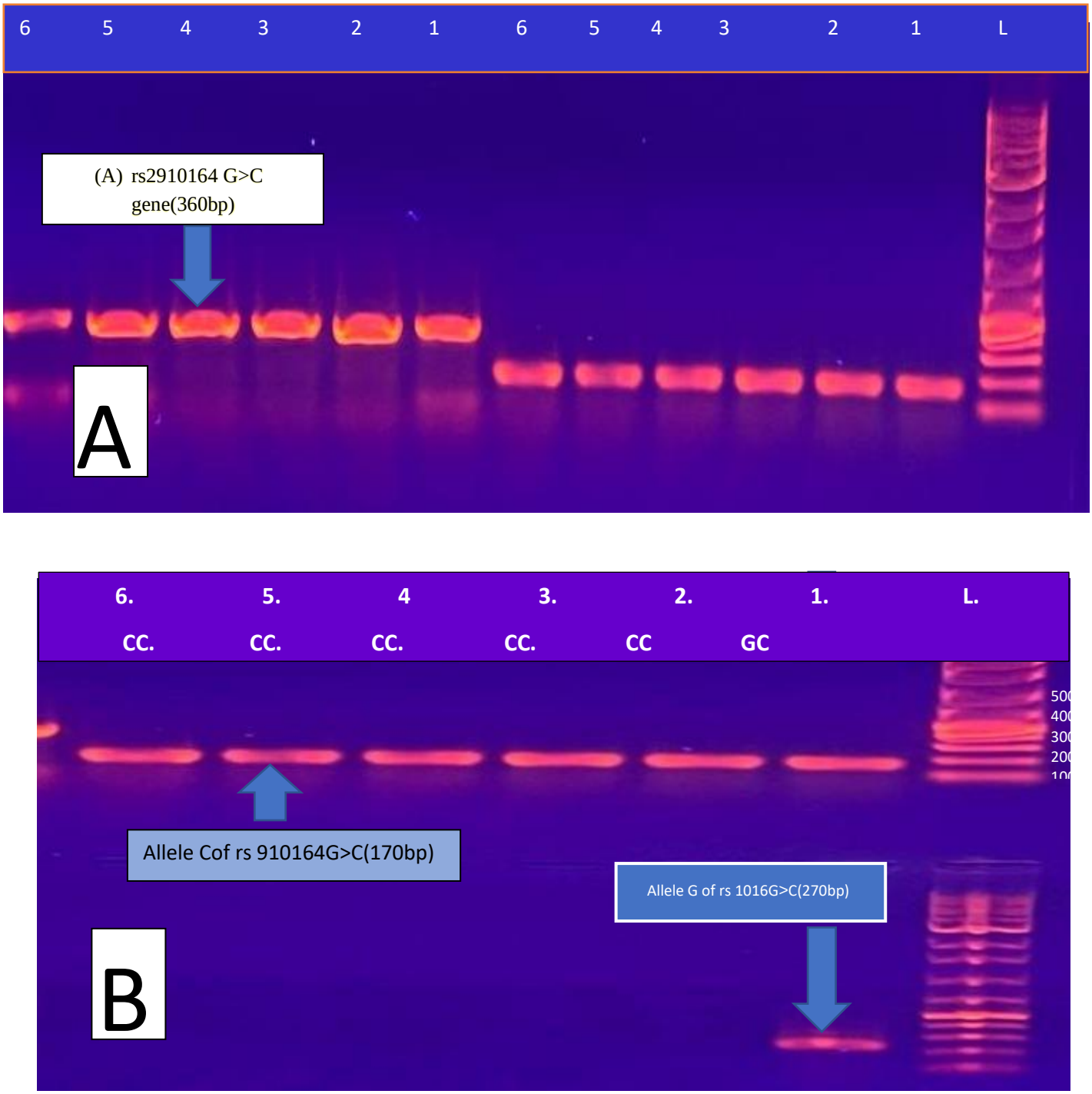


Figure (2) Agarose gel electrophoresis (1.5%, 1x TBE) showing PCR product of rs2910164 G>C for alleles C(170bp) by using RO&FI as primers(A &B) and G(270bp) by using FO&FI by an allele-specific PCR assay in RA patients. (B)Lane 1 represented the genotype GC, while the lane 2,3,4,5,6, showed the genotype CC. Agarose gel at 80% volts for one hour, Lane L: 100-1500 bp ladder

- Molecular Assessment of rs2910164G>Cgene Polymorphism in UTI patients

In UTI patients the genotype and allele frequency of rs2910164G>Cgene polymorphism are shown in table (3) and figure (2). All genotypes of this gene (GG, GC, CC)

rs2910164G>Cgene polymorphism displayed a non-significant association (P≤0.05) in comparison with the control group. In addition to that, allele G rs2910164G>Cgene had a high ratio in UTI (**77.33**%) in comparison with the control group (**68.12**). In contrast, allele C rs2910164G>Cgene had a high ratio in the control group (31.87%) in comparison with the UTI group(**22.67**%) as in table (3).

The frequency distribution of rs2910164 G>C genotypes in UTI patients and normal control group are demonstrated that polymorphism of rs2910164 G>C gene was non-significant risk factor for predisposition to RA .Furthermore, the rs2910164 G>C C allele was identified as non-risk factor for susceptibility to RA in UTI (OR, 1.595; 95% CI, 0.961-2.649; P=0.070) as in table (3).

Table(3): Distribution of genotypes (GG, GC, CC) and alleles (G, C) of the rs2910164 G>C gene in UTI patients .

Genotype/ allele	UTI (No. =75)	Control(No.= 80)	P. value	OR	
Genotype					
GG	**46**	**42**	**0.863(NS)**	**1.048**	**0.613 to 1.791**
GC	**24**	**25**	**0.920(NS)**	**1,035**	**0.525 to 2.038**
CC	**5**	**13**	**0.070(NS)**	**0,3681**	**0.124 to 1.088**
Allele					
G	**116(77.33%)**	**109 (68.12%)**	**0.070**	**1.595**	**0.961 to 2.649**
C	**34 (22.67%)**	**51(31.87%)**			

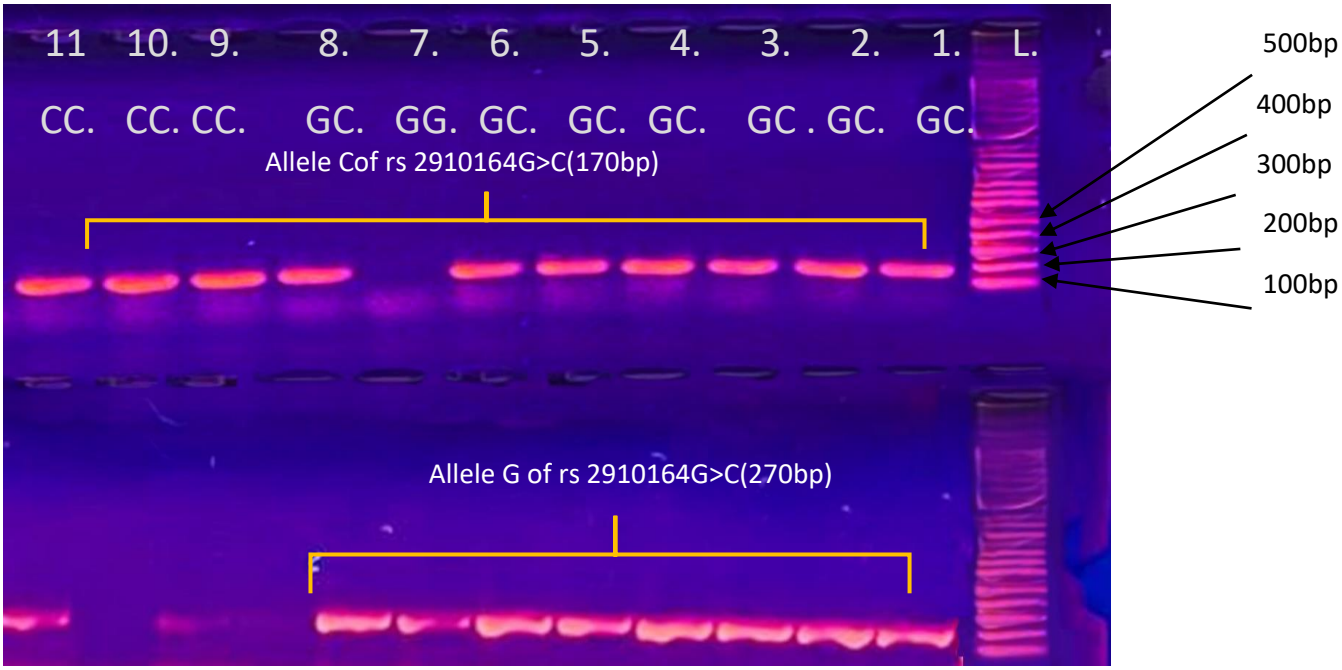


Figure (3) Agarose gel electrophoresis (1.5%, 1x TBE) showing PCR product of rs2910164 G>C for alleles C(170bp) by using RO&FI as primers and G(270bp) by using FO&RI by an allele-specific PCR assay in UTI patients. Lane 1,2,3,4,5,6,8 represented the genotype GC, while the lane 7represented the genotype GG and Lane9,10,11 showed the genotype CC. Agarose gel at 80% volts for one hour, Lane L: 100-1500 bp ladder.

-Comparison the distribution of the genotypes (GG, GC, CC) and alleles (G, C) of the rs2910164 G>C gene among UTI and RA patients are showing in

Table(4-6): Comparison the distribution of the genotypes (GG, GC, CC) and alleles (G, C) of the rs2910164 G>C gene among UTI and RA patients.

Genotype/ allele	UTI (No. =75)	RA(No.= 70)	P. value	OR	C.I 95%
Genotype					
GG	46	34	0.123	1.679	0.868 to 3.249
GC	24	10	0.013(S)	2.823	1.235 to6.454
CC	5	26	0.0001(S)	0.120	0.043 to 0.338
Allele					
G	116(77.33%)	78(55.74%)	0.0001(S)	2.711	1.633 to 4.503
C	34 (22.67%)	62(44.27%)			

In the present study, we analyzed the correlation between genetic polymorphisms in rs2910164 gene and susceptibility to RA in RA and in relation to UTI patients. The rs2910164 polymorphism was revealed to be associated with an overall increased risk of RA. The prevalence of genotype CC (26) variants in RA patients were identified as significantly higher than that in the UTI(5) and healthy individuals (13) and the C allele (minor allele) of rs2910164 was found to be as more frequent in patients with RA than that of UTI and controls (44.27,22.67,31.87%, respectively). In this study, An association was detected between rs2910164 gene polymorphism and the risk of RA.

In contrast to present findings, other Iraqi study (Dlshad& Tahir,2022) has shown a marked correlation between the mRNA 499 rs3746444 polymorphism and susceptibility to RA in the samples of the Erbil population and, no association was revealed between Mrna146ars2910164 variant and RA susceptibility. Regarding pre-miRNA-146a rs2910164 polymorphism, show GC and CC genotypes are risk factors for **RA** with (OR= 1.185 and 1.077)respectively, but there is no **significant** correlation as (P value = 0.681) for this gene.However, the authors observed that carriers of the CT genotype in rs3746444 had a higher level of anti-cyclic citrullinated protein (CCP) antibody. In a previous Iranian study(Hashemi et al.,2013) has shown a marked correlation between the hsa-mir-499 rs3746444 polymorphism and susceptibility to RA in a sample of the Iranian population. However, no association was revealed between the hsa-mir-146a rs2910164 variant and RA susceptibility. They demonstrated that mi RNA polymorphisms may be suitable for use as diagnostic biomarkers

for RA in future. Also the presnt results di agreement with Khan et al.(2022) Who reported no association was found between rs2910164 and predisposition to RA in our population.

Many previous studies (Guo et al.,2019 & Doghishet al.,2022) have revealed that polymorphisms in miRNA target sites affect the pathogenesis of several human diseases. Polymorphisms in miRNA genes may alter a wide spectrum of biological processes by affecting the processing and/or target selection of miRNAs. Cezar-de-Mello et al. (2014) reported that miRSNP-146a was the unique gene associated with risk to leprosy per se (GC OR = 1.44, p = 0.04; CC OR = 2.18, p = 0.0091). further that they found the C-allele was over-transmitted (p = 0.003) using a transmission disequilibrium test and MiR-146a is known to modulate TNF levels.

In conclusion, the present study has shown a marked correlation between the rs2910164 polymorphism and susceptibility to RA in the samples of the Najaf population.However, no association was revealed between rs2910164 polymorphism and UTI and GG genotype is the dominant type ,according to this genetic results,UTI could not conceder predisposing to cause RA . Consistent with growing evidence, the present study has demonstrated that miRNA polymorphisms may be suitable for use as diagnostic biomarkers for RA infuture.

Reference

1) Abdul, F. R., Raheem, I. A., Subhi, H. T., Fatah, H. R., & Muhammad, H. A. (2023). Immunological parameters of Proteus mirabilis isolates in Rheumatoid Arthritis patients with urinary tract infections. bioRxiv, 2023-01.
2) Al-Hamdani, H., & Al-Hashimy, A. (2020). Molecular detection of UREC, HPMA, RSBA AND MRPA genes of Proteus Mirabilis urinary tract infection in patient with rheumatoid arthritis. The Iraqi Journal of Agricultural Science, 51, 245-251.
3) Bartolomé-Casado, R., Landsverk, O. J., Chauhan, S. K., Sætre, F., Hagen, K. T., Yaqub, S., ... & Jahnsen, F. L. (2021). CD4+ T cells persist for years in the human small intestine and display a TH1 cytokine profile. Mucosal Immunology, 14(2), 402-410.
4) Cezar-de-Mello, P. F., Toledo-Pinto, T. G., Marques, C. S., Arnez, L. E., Cardoso, C., Guerreiro, L. T., ... & Moraes, M. O. (2014). Pre-miR-146a (rs2910164 G> C) single nucleotide polymorphism is genetically and functionally associated with leprosy. *PLoS neglected tropical diseases*, *8*(9), e3099.
5) Chauhan, K., Jandu, J. S., Goyal, A., & Al-Dhahir, M. A. (2021). Continuing Education Activity. StatPearls [Internet]; Treasure Island (FL): StatPearls Publishing: Mountain View, CA, USA.
6) Dlshad, M., & Tahir, F. (2022). Association of pre-miRNA-146a rs2910164 and pre-miRNA-499a rs3746444 polymorphisms with Rheumatoid arthritis in Erbil province.
7) Doghish, A. S., Ismail, A., El-Mahdy, H. A., Elkhawaga, S. Y., Elsakka, E. G., Mady, E. A., ... & El-Husseiny, H. M. (2022). miRNAs insights into rheumatoid arthritis: Favorable and detrimental aspects of key performers. *Life Sciences*, 121321.
8) Guo, Y., Hong, W., Wang, X., Zhang, P., Körner, H., Tu, J., & Wei, W. (2019). MicroRNAs in microglia: how do microRNAs affect activation, inflammation, polarization of microglia and mediate the interaction between microglia and glioma? *Frontiers in molecular neuroscience*, *12*, 125.
9) Hao, D., Wang, X., Yang, Y., Chen, H., Thomsen, B., & Holm, L. E. (2023). MicroRNA sequence variation can impact interactions with target mRNA in cattle. Gene, 868, 147373.
10) Hashemi, M., Eskandari-Nasab, E., Zakeri, Z., Atabaki, M., Bahari, G., Jahantigh, M., ... & Ghavami, S. (2013). Association of pre-miRNA-146a rs2910164 and pre-miRNA-499 rs3746444 polymorphisms and susceptibility to rheumatoid arthritis. *Molecular medicine reports*, *7*(1), 287-291.

11) Khan, R., Abbasi, S. A., Mansoor, Q., Ahmed, M. N., Mir, K. B., & Baig, R. M. (2022). Analysis of Rare Alleles of miRNA-146a (rs2910164) and miRNA-34b/c (rs4938723) as a Prognostic Marker in Thyroid Cancer in Pakistani Population. *Diagnostics*, *12*(10), 2495.
12) Kogo R, Mimori K, Tanaka F, Komune S, Mori M (2011) Clinical significance of miR-146a in gastric cancer cases. Clin Cancer Res 17: 4277–4284.
13) Kwon, E. J., & Ju, J. H. (2021). Impact of posttranslational modification in pathogenesis of rheumatoid arthritis: Focusing on citrullination, carbamylation, and acetylation. International journal of molecular sciences, 22(19), 10576.
14) Li, K., Tie, H., Hu, N., Chen, H., Yin, X., Peng, C., ... & Huang, W. (2014). Association of two polymorphisms rs2910164 in miRNA-146a and rs3746444 in miRNA-499 with rheumatoid arthritis: a meta-analysis. Human immunology, 75(7), 602-608.
15) Mo, X., Guo, Y., Qian, Q., Fu, M., & Zhang, H. (2020). Phosphorylation-related SNPs influence lipid levels and rheumatoid arthritis risk by altering gene expression and plasma protein levels. Rheumatology, 59(4), 889-898.
16) Shandilya, U. K., Wu, X., McAllister, C., Mutharia, L., & Karrow, N. A. (2023). Role of Toll-Like Receptor 4 in Mycobacterium avium subsp. paratuberculosis Infection of Bovine Mammary Epithelial (MAC-T) Cells In Vitro. *Microbiology Spectrum*, *11*(2), e04393-22.
17) Wang, X., Xing, Y., Wang, Y., Du, Z., Zhang, C., & Gao, J. (2023). Association of microRNA gene polymorphisms with recurrent spontaneous abortion: An updated meta-analysis. *Experimental and Therapeutic Medicine*, *25*(4), 1-15.
18) Welle J.M. (2010). Textbook of Urinalysis and Body Fluids: A Clinical Approach. Appleton-Century-Crofts; first edition.UK.
19) Xu B, Feng N-H, Li P-C, Tao J, Wu D, et al. (2010) A functional polymorphism in pre-miR-146a gene is associated with prostate cancer risk and mature miR146a expression in vivo. The Prostate 70: 467–472. 63.
20) Zhang, J., Fang, X. Y., Wu, J., Fan, Y. G., Leng, R. X., Liu, B., ... & Ye, D. Q. (2023). Association of Combined Exposure to Ambient Air Pollutants, Genetic Risk, and Incident Rheumatoid Arthritis: A Prospective Cohort Study in the UK Biobank. Environmental Health Perspectives, 131(3), 037008.
21) Chandan, K., Gupta, M., & Sarwat, M. (2020). Role of host and pathogen-derived microRNAs in immune regulation during infectious and inflammatory diseases. Frontiers in immunology, 10, 3081.