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Association of BIRC5 (Survivin) Gene Polymorphisms (rs9904341 and rs17878467) and mRNA Expression with Rheumatoid Arthritis Susceptibility and DMARD Response in Iraqi Patients

Da·ad A. Hussain[1*], Asmaa Mhmood Salman[2], Sarah Alauladdine Abdulhameed[3]

[1] Institute Genetic Engineering and Biotechnology for postgraduate Studies, Baghdad University, Baghdad, Iraq.

[2] Department of Human Anatomy, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq.

[3]Institute Genetic Engineering and Biotechnology for postgraduate Studies, Baghdad University, Baghdad, Iraq.

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is both genetically and environmentally determined. The objectives of the present study were to test the relationship between two single nucleotide polymorphisms (SNPs) of BIRC5 (Survivin) gene-rs9904341 and-rs17878467 and RA susceptibility and response to disease-modifying anti-rheumatic drugs (DMARDs) in Iraqi patients. A total of ~~90~~70 RA patients and 30 age- and sex-matched healthy controls were sampled in terms of blood. Taquinase-associated genotyping was carried out and mRNA expression was determined by using qRT-PCR.
RA patients exhibited a significant higher prevalence of the rs9904341 C allele and but not with the CC genotype and were associated with an increased disease activity. In the same way, the RA had a significant association with the genotype, rs17878467 AA. It is interesting to note that these two SNPs demonstrated a different distribution pattern, between patients who were treated with methotrexate alone, and those who were treated with biologic therapy. Additionally, BIRC5 mRNA levels were also highly expressed in RA patients than controls and most importantly, patients on methotrexate monotherapy. The presented findings indicate that the BIRC5 gene polymorphs and expression levels can be considered possible genetic and molecular biomarkers of RA predisposition and treatment stratification among Iraqis.

Keywords
Rheumatoid arthritis, BIRC5, Survivin, rs9904341, rs17878467, gene expression, DMARD therapy.

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovium, which ends in joint erosion, deformity, and permanent disability. It impacts about 0.5-1 per cent. of the entire population on the earth, and its prevalence is much greater among females. Despite evading its definite etiopathogenesis, RA arises on a spectrum of multifactors such as genetic susceptibility, environmental precipitants and immune dysregulation (1).

Corresponding Author: Da,ad A. Hussain, E-mail: daad@ige.uobaghdad.edu.iq

Over the last several years, much effort has been paid to the importance of apoptotic failure in the pathogenesis of RA. It is believed that synovial hyperplasia, the formation of pannus, and the deposition of immune cells, in a way, is caused due to the disrupted process of apoptosis. A protein that has been identified to play a major role in this process is survivin, a protein coded by BIRC5 gene. Survivin is a protein, which is on chromosome 17q25 and is an inhibitor of apoptotic proteins (IAP) and is involved with the inhibitory effect of cell death and the control of mitosis (2).

The increased levels of survivin have been reported in synovial tissues, peripheral blood mononuclear cells (PBMCs) and the serum of RA patients. Interestingly, its existence is associated with increased disease activity, greater radiological damage and poor prognosis (3, 4). Although its extensive investigation is performed in the oncology field, survivin is currently developing as a biomarker in autoimmune diseases like RA.

One of the mechanisms that regulate the expression of survivin is genetic polymorphism of the BIRC5 promoter. Two of them, which are rs9904341 (-31G C) and rs17878467 (-241C T), have been shown to change transcription of genes by changing the binding sites of transcription factors (5) (6). Several studies have linked the presence of the susceptibility cancers and survivor overexpression with rs9904341; this has not been well studied in reference to autoimmune diseases; especially RA. The role of rs17878467, in turn, is even less developed, though there are certain signs that it might be involved in the pathogenesis of autoimmunity (7).

At the same time, survivin as a therapeutic response predictor was investigated recently. DMARDs have been the main pillars of RA treatment, although not every patient can reach satisfactory remission. Discovery of molecular biomarkers predictive of therapeutic outcome may be used to tailor individualized therapy and enhance disease management (8).

So far, there has been no research done to investigate the connection between BIRC5 promoter polymorphisms (rs9904341 andrs17878467), survivin mRNA expression and DMARD response among the Iraqi population. The explanation of these associations could provide important information about the genetic and molecular basis of RA and guide more specific treatment approaches. To our knowledge, this is the first study to simultaneously evaluate BIRC5 promoter polymorphisms, survivin mRNA expression, and DMARD treatment response within a single Iraqi RA cohort, an integrated approach that adds value beyond prior studies examining these endpoints separately.

Objectives

The purpose of the study will be to evaluate the presence of differences in BIRC5 gene at both genetic and transcriptional levels in pathogenesis of rheumatoid arthritis (RA), as well as to determine whether the variations can predict the response to disease-modifying antirheumatic drugs (DMARDs) in Iraqi patients. In particular, the research will aim to:

1. Identify the relationship between BIRC5 promoter polymorphism (rs9904341 and rs17878467) and RA.
2. Determine the BIRC5 mRNA levels of expression in the peripheral blood of RA patients and other healthy people.
3. Measures the BIRC5 gene expression relative to treatment response in patients receiving conventional and biologic DMARDs.
4. Determine the relationship between particular genotypes with altered survivin expression or clinical surrogates of RF and CRP levels.

Materials and Methods

The study was a case-control study and was approved by the ethical committee of the Institute of Genetic Engineering and Biotechnology, the University of Baghdad (approval number 3143, dated 4/4/2025). This was a combination of both molecular and clinical studies endeavored to explain the association between Survivin (BIRC5) gene expression and promoter polymorphisms (rs9904341 and rs17878467) and susceptibility to rheumatoid arthritis in addition to their effects on therapeutic response.

Subjects

total of **100 individuals** were enrolled in this study:

- **70 patients diagnosed with RA** (14 males, 56 females)
- **30 apparently healthy controls** (15 males, 15 females)

Patients were recruited from **Baghdad Teaching Hospital** and **Al-Yarmouk Teaching Hospital** between April and August 2025. Participants ranged in age from **22 to 75 years**. All subjects signed informed consent prior to participation.

Patients were receiving different treatments:

- **35 patients were on chemotherapy**
- **35 patients were on biological therapy**

Diagnosis of RA was confirmed based on **ACR/EULAR 2010 criteria**, and patients with other autoimmune diseases or cancer were excluded.

2. Sample Collection and Preparation

- **5 mL of venous blood** was collected from each subject.
  - **2 mL into EDTA tubes** for DNA and RNA extraction.
  - The remaining was used to isolate serum for RF and CRP testing.
- **TRIzol® LS reagent** was used to preserve RNA at a 3:1 ratio (TRIzol:blood), then stored at –23 °C overnight.

1. **RNA Extraction and Gene Expression Analysis (qRT-PCR)**

RNA Extraction

- Performed using **TransZol Up Plus RNA Kit** (TransGen, China).
- The procedure included:
  - Phase separation with chloroform.
  - RNA precipitation with ethanol.
  - Column-based purification and elution with RNase-free water.
- RNA concentration and purity were assessed using a **Nanodrop 2000c**, measuring A260/A280 ratios.

cDNA Synthesis

- Done using the **EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix**.
- Reaction mix included random primers and oligo(dT)18.
- Reverse transcription conditions:
  - 25 °C for 10 min (primer annealing)
  - 42 °C for 15 min (extension)
  - 85 °C for 5 sec (enzyme inactivation)

qRT-PCR

- Target gene: **BIRC5 (Survivin)**
- Housekeeping gene: **GAPDH**
- Performed using **QIAGEN Rotor-Gene Q** system with **TransStart® Top Green qPCR Super Mix**.
- Thermal profiles:
  - **BIRC5**: 94 °C (30s), 40 cycles of 94 °C (5s), 60 °C (15s), 72 °C (20s)
  - **GAPDH**: same profile but annealing at 58 °C
- Expression levels were calculated using the **2^-ΔΔCt method**.

2. **Genomic DNA Extraction and SNP Genotyping**

DNA Extraction

- Performed using **EasyPure® Blood Genomic DNA Kit** (TransGen, China).
- Involved lysis, RNase/proteinase K treatment, ethanol precipitation, and spin column purification.
- DNA quality assessed via **Nanodrop 2000c** and **agarose gel electrophoresis (1%)**.

HRM Genotyping

- SNPs **rs9904341** and **rs17878467** were genotyped using **High Resolution Melting (HRM) analysis**.
- Performed on the **QIAGEN Rotor-Gene Q** with **TransStart® Tip Green qPCR Super Mix**.
- Primer sequences for both SNPs were designed using Primer3 and validated via UCSC and NCBI databases.
- Melting curves analyzed using Rotor-Gene software with HRM Tool.

The primers used for BIRC5 mRNA expression and genotyping of rs9904341 and rs17878467 polymorphisms were designed using Primer-BLAST The sequences and product sizes are listed in Table 1.

Table 1: primers sequences.

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta °C
BIRC5 (Survivin)				

Forward	**GAGGCTGGCTTCATCCACT**	**24**	**93**	**60**
Reverse	**ATCTGGCTCCCAGCCTTC**	**20**		
GAPDH- Glyceraldehyde 3-phosphate dehydrogenase				
Forward	**GAAATCCCATCACCATCTTCCAGG**	**24**	**101**	**58**
Reverse	**GAGCCCCAGCCTTCTCCATG**	**20**		
rs17878467				
Forward	**CGCGTTCTTTGAAAGCAGTCG**	**21**	**60**	**58**
Reverse	**CGCCAGCTCGTCCCTGCC**	**18**		
rs9904341				
Forward	**CGCCATTAACCGCCAGATTTGAA**	**23**	**51**	**58**
Reverse	**CCGCCACCTCTGCCAACG**	**18**		

5. Statistical Analysis

- Data were analyzed using **SPSS v29** and **GraphPad prism 9**.
- **Quantitative data**: presented as mean ± SD.
- **Qualitative data**: presented as frequencies and percentages.
- Tests applied:
- **ANOVA** followed by **Duncan's post hoc test** for group comparisons.
- **Chi-square test** for categorical variables.
- **Fisher's exact test** and **Odds Ratios** were calculated using WINPEPI v11.63.
- Significance thresholds:
- $P \leq 0.05$: Significant
- $P \leq 0.01$: Highly Significant.

Results

Demographic and Clinical Characteristics

Table 2 outlines the demographic and clinical characteristics of the people that will be divided into three different groups: the chemotherapy group with 35 participants, the biotherapy group with 35 people and the control group with 30 people.

Table 2: Demographic and clinical characteristics of patients and Control.

Characteristics	**Chem therapy (no.35)**	**Bio Therapy (no.35)**	**Control (no.30)**
Age (years) Mean ± SD	49.11±9.322	45.94± 9.849	46.17± 13.527
p-value	**0.4 NS**		
BMI Mean SD	28.66±5.45	28.71±4.417	26.59± 3.11
p-value	**0.1 NS**		
Sex Male (no) Female (no)	5 (14%) a 30 (86%) a	9 (26%) b 26 (74%) b	15 (50%) b 15 (50%) b
p-value	**0.006****		
ESR Mean SD	33.40 ±10.40 a	32.85±11.36 a	12.00± 4.242 b

p-value	**0.001 ****		
RF **Negative** **Positive**	14 (40%) a 21 (60%) a	20 (57%) a 15 (43%) a	26 (87%) b 4 (13%) b
p-value	**0.001****		
CRP **Mean SD**	10.180 ±9.50 a	11.585±10.92 a	3.967 ± 1.21 b
p-value	**0.007****		

Data were expressed as mean ± SD. Statistical analyses were performed by ANOVA followed by Post Hoc test (Duncan's test) for multiple comparisons. ANOVA significance test (2-tailed). Significant values are bolded. a and b: Different letters mean there is significant difference. Means followed by the same letter are not significantly different. SD: Std. Deviation, SE: Std. Error of Mean, NS, no significant difference. *: Significant (P≤0.05). **: Highly Significant (P≤0.01).
Statistical analyses performed by the Chi-squared test; NS, no significant difference; Significant values are bolded. Each subscript letter denotes a subset of Groups categories whose column proportions do not differ significantly from each other at the .05 level.

The results of the analysis did not show any statistically significant differences in the means of age in the three groups (Chem: 49.11 ± 9.32; Bio: 45.94 ± 9.85; Control: 46.17 ± 13.52; p=0.4) or in body mass index (Chem: 28.66 0.54: Bio: 28.71 0.44: Control:
An observable statistically significant difference in Sex distribution was noted (p= 0.006) where the percentage of females in both treatment groups (Chem: 86% and Bio: 74% each) was significantly higher than the percentage of the control group (50%).
The sedimentation rate of erythrocytes (ESR) was significantly higher in the chemotherapy (33.4020 means9.401015mm/hr) and biotherapy (32.851136mm/hr) groups compared to the controls (12.00424mm/hr), and its increase was highly significant (p=.001).
On the same note, rheumatoid factor (RF) positivity was significantly higher in patients receiving biotherapy (60%), and chemotherapy (43%), compared to the controls (13%), with the differences having a highly significant statistical threshold (p = 0.001).
The CRP levels were significantly higher in both methotrexate-treated and biologic-treated RA patients (10.18 ± 9.50 mg/L and 11.58 ± 10.92 mg/L, respectively) compared to the control group (3.97 ± 1.21 mg/L) (p = 0.007). The values marked with different superscripts (a, b) indicate statistically significant differences between the groups.
Taken altogether, the findings indicate that the clinical characteristics in the intervention groups is related to active autoimmune pathology, specifically in inflammatory biomarkers of ESR, RF and CRP and is equal to the control group in terms of age and BMI.

Expression Levels of BIRC5 mRNA
Statistically significant inter-group difference in the abundance of BIRC5 transcripts was revealed by qRT-PCR assessment in real time (figure 1,2). Table 3 shows the mean values of cycle threshold (Ct) of BIRC5, and it shows that there is a slight increase in chemotherapy cohort of BIRC5 (22.554) compared to bio-therapy (21.454) and control cohort (21.264).
When normalized to GAPDH and analyzed using the 2^-ΔΔCt method, the fold change in gene expression revealed a significant downregulation of BIRC5 in the chemotherapy group (0.411-fold; p = 0.001), which is also consistent with the activity of survivin being attenuated after cytotoxic exposure. On the contrary, bio-therapy cohort showed an almost intact expression level (0.970 -fold) with no statistically significant difference with the control group.

Table 3: gene expression of gene *BIRC5.*

groups	***Means Ct of BIRC5***	***Means Ct of GAPDH***	***ΔCt (Means Ct of BIRC5)***	Δct cal	ΔΔCT	$2^{-\Delta\Delta CT}$	***experimental group/ Control group***	***Fold of gene expression***	*p-value*

Chem therapy	22.554	25.120	-2.566	1.2	-3.766	13.604	13.604/33.106	0.411 b	0.001*
Bio Therapy	21.454	25.26	-3.806	1.2	-5.006	32.133	32.133/33.106	0.970 a	
Control	21.264	25.113	-3.849	1.2	-5.049	33.106	33.106/33.106	1.000 a	

Relative gene expression levels were calculated using the comparative Ct method (2^-ΔΔCt) described by Livak and Schmittgen (2001).

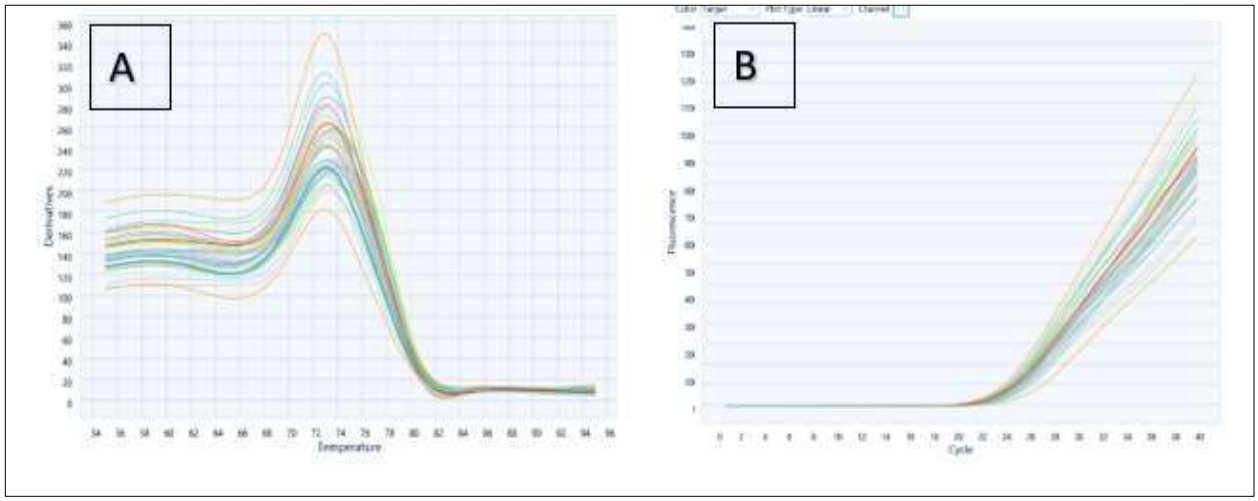


Figure 1: Glyceraldehyde-3-phosphate dehydrogenase: (a) Melting curve analysis of GAPDH (b) the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly.

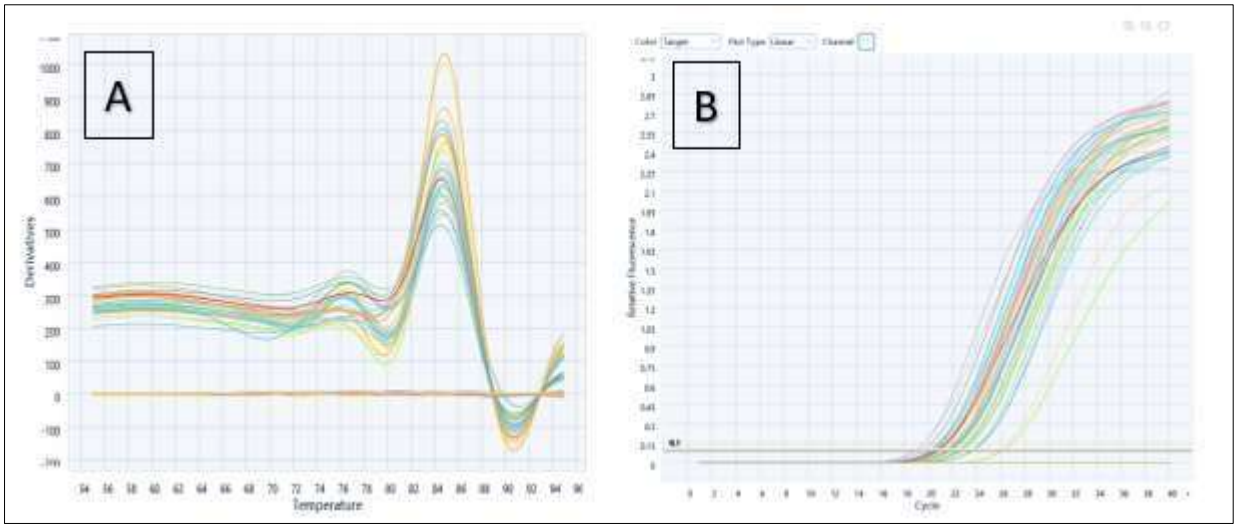


Figure 2: *BIRC5*: (a) Melting curve analysis (b) the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly.

The results suggest that chemotherapeutic interventions have repressive effect on the BIRC5 transcription, and that biologically directed treatment does not produce a similar effect on this gene.

Genotype Distribution and Hardy-Weinberg Equilibrium

Table 4 lists the actual and the theorized genotype frequencies of SNP = rs9904341 in the three different cohorts (Control, Chemotherapy and Biotherapy), and the Hardy Weinberg equilibrium p-values.

Table 4: The percentage frequencies of genotype and Hardy-Weinberg Equilibrium (HWE) of rs9904341 in the studied groups.

Groups	rs9904341		GG	GA	AA	HWE P>0.05
Controls (No.30)	Observed	N	15	14	1	0.3 NS
	Expected	N	16.133	11.733	2.133	
Chem therapy Gr. (No.35)	Observed	N	2	1	32	0.9 NS
	Expected	N	0.179	4.643	30.179	
Bio Therapy Gr. (No.35)	Observed	N	1	14	20	0.4 NS
	Expected	N	1.829	12.343	20.829	

The genotype distribution in the control group was GG (50%), GA (46.7%) and AA (3.3%); there was no deviation from HWE and p-value=0.3). In the chemotherapy group, however, significantly predominance of AA genotype was shown (91.4%), and GG and GA genotypes were marginal rare (5.7% for both), respectively, but here also weighted population did not showed significant deviation from HWE (p=0.9). The biotherapy group showed a modest distribution with GG (2.9%), GA (40%) and AA (57.1%), also with no significant deviation from HWE (p=0.4).

Despite the equilibrium suggesting random mating within the population sample, the skewed distribution, especially the predominance of the AA genotype in the chemotherapy group, may suggest a potential association between rs9904341 and the response to chemotherapy.

Additionally, the genotype frequencies for SNP rs17878467 and HWE computations for the same study groups are shown in Table 5.

Table 5: The percentage frequencies of genotype and Hardy-Weinberg Equilibrium (HWE) of rs17878467 in the studied groups.

Groups	rs17878467		CC	CG	GG	HWE P>0.05
Controls (No.30)	Observed	N	6	17	7	0.5 NS
	Expected	N	7.008	14.983	8.008	
Chem therapy Gr. (No.35)	Observed	N	27	4	4	0.01**
	Expected	N	24.029	9.943	1.029	
Bio Therapy Gr. (No.35)	Observed	N	19	15	1	0.3 NS
	Expected	N	20.064	12.871	2.064	

The genotype frequencies in control group were: CC (20%), CG (56.7%) and GG (23.3%) without any deviation to HWE (p=0.5). In the chemotherapy group, the representation of CC genotype (77.1%) was significantly higher than CG (11.4%) and GG (11.4%) with significant HW disequilibrium also observed (p=0.01), implicating selective pressure or genetic association in chemotherapy patients. The biotherapy group showed a relatively balanced genotype distribution: CC (54.3%), CG (42.9%) and GG (2.9%) and the population was in HWE (p=0.3).

The significant difference in the chemotherapy group for rs17878467 might indicate that it plays a part in determining therapeutic results or susceptibility when subjected to chemotherapeutic pressure.

Genotype and Allelic Associations

As shown in Table 6, a significant difference was observed in the distribution of rs9904341 genotypes and alleles between patients and controls (Figure 3).

Table 6: Genotype and allele frequencies detected by hardy-Weinberg equilibrium law of rs9904341 gene polymorphism SNP between patient group and control group.

rs9904341	Control (No.30)	Chem therapy Gr. (No.35)	Bio Therapy Gr. (No.35)	OR* (CI:95%)	OR** (CI:95%)	P value*	P value**

GG	15 (50%)	2 (5.7%)	1 (2.9%)	16.5 (3.34-81.45)	34.4 (4.10-281.41)	**0.0006**	**0.001**
GA	14 (47%)	1 (2.9%)	14 (40%)	27.75 (3.59-246.3)	1.3 (0.48- 3.51)	**0.001**	**0.6 NS**
AA	1 (3%)	32 (91.4%)	20 (57.1%)	32.2 (3.0-278.9)	25.6 (3.2-211.4)	**0.0001**	**0.006**
G	44 (73.3%)	5 (7%)	16 (23%)	35.7 (12.20-104.70)	9.2 (4.17-20.63)	**0.0001**	**0.0001**
A	16 (26.7%)	65 (93%)	54 (77%)				

*(Chem therapy Gr. And Control), **(Bio Therapy Gr. And Control).

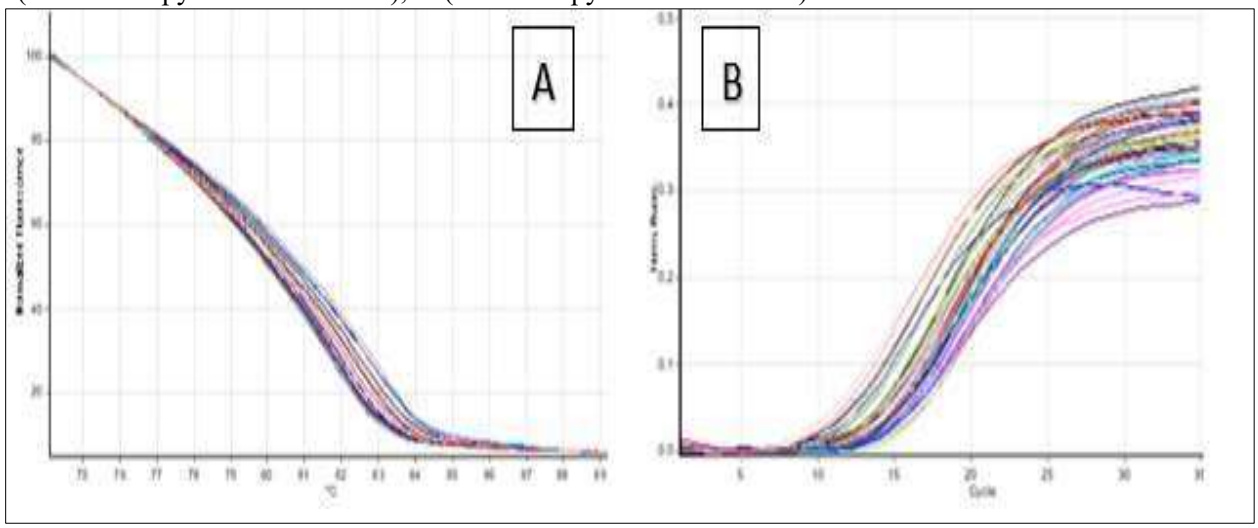


Figure 3: rs9904341: (a) HRM plot (b) the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly.

Compared to just 3% in the control group, the AA genotype was significantly more common in the chemotherapy group (91.4%) and the biotherapy group (57.1%) ($p = 0.0001$ for Chem vs. Control; $p = 0.006$ for Bio vs. Control). On the other hand, the GG genotype was almost nonexistent in the groups receiving biotherapy (2.9%) and chemotherapy (5.7%) but dominant in the control group (50%) as well. There was a highly significant inverse association (OR = 27.75, 95% CI: 3.59–246.3; $p = 0.001$) between the GA genotype and the need for chemotherapy, with the GA genotype being present in 47% of the control group and almost nonexistent in the chemotherapy group (2.9%). Intriguingly, the biotherapy group's GA frequency stayed at a comparatively high 40%, and there was no discernible difference when compared to the controls (OR = 1.3, 95% CI: 0.48–3.51; $p = 0.6$), suggesting that there isn't a strong correlation in this situation.

Compared to 26.7% in controls, the A allele was substantially more common in both patient groups (93% in chemotherapy and 77% in biotherapy) ($p = 0.0001$ for both comparisons). Strong allelic association was indicated by the A allele's odds ratio (OR) of 35.7 (95% CI: 12.20–104.70) in the chemotherapy group and 9.2 (95% CI: 4.17–20.63) in the biotherapy group when compared to the control group. The significant allelic shift linked to disease status and treatment response was further supported by the fact that the G allele was nearly absent in both patient groups (7% in chemotherapy and 23% in biotherapy) but dominant in the control group (73.3%). These results suggest that the A allele and AA genotype of rs9904341 are significantly associated with rheumatoid arthritis in the studied Iraqi cohort.

In Table 7, rs17878467 also showed significant differences in genotype and allele frequencies between groups (Figure 4):

Table 7: Genotype and allele frequencies detected by hardy-Weinberg equilibrium law of rs17878467 gene polymorphism SNP between patient group and control group.

rs17878467	Control (No.30)	Chem therapy Gr. (No.35)	Bio Therapy Gr. (No.35)	OR* (CI:95%)	OR** (CI:95%)	P value*	P value**
CC	6 (20%)	27 (77.2%)	19 (54%)	47.2 (22.5-244.3)	21.5 (6.91-64.18)	**0.0001**	**0.006**
CG	17 (57%)	4 (11.4%)	15 (43%)	10.1 (2.85-35.98)	1.7 (0.65- 4.66)	**0.0003**	**0.2 NS**
GG	7 (23%)	4 (11.4%)	1 (2.9%)	2.3 (0.61-9.02)	10.3 (1.19-89.82)	**0.2 NS**	**0.03**
C	29 (48%)	58 (83%)	53 (76%)	5.1 (2.31-11.51)	3.3 (1.58-7.01)	**0.0001**	**0.001**
G	31 (52%)	12 (17%)	17 (24%)				

*(Chem therapy Gr. And Control), **(Bio Therapy Gr. And Control)

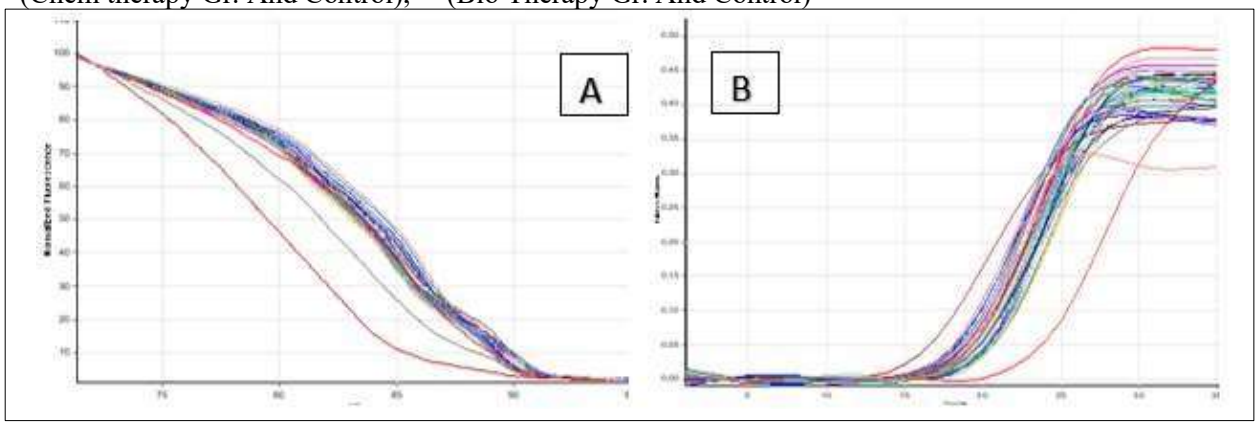


Figure 4: rs17878467: (a) HRM plot (b) the amplification plots by quantitative polymerase chain reaction (qPCR) samples. The Qiagen Rotor-Gene Q 6000 qPCR machine was used to take the picture directly.

Compared to just 20% in the control group, the CC genotype was significantly more prevalent in the chemotherapy group (77.2%) and the biotherapy group (54%), respectively (p = 0.0001 and 0.006). With p = 0.0003 and OR: 10.1 (95% CI: 2.85–35.98), the CG genotype revealed a significant difference between the chemotherapy group (11.4%) and the control group (57%). For this genotype, however, there was no discernible difference between the biotherapy and control groups (p = 0.2). Just 2.9% of the biotherapy group had the GG genotype, compared to 23% of the control group and 11.4% of the chemotherapy group. It was significantly lower in biotherapy patients than in controls (p = 0.03; OR: 10.3, 95% CI: 1.19–89.82), but not significant in Chem vs. Control (p = 0.2).

In terms of allele frequencies, patients had a significantly higher frequency of the C allele (83% in chemotherapy and 76% in biotherapy) than controls (48%; p = 0.0001 and 0.001, respectively). The G allele, on the other hand, was significantly less common in the patient groups (17% in chemotherapy and 24% in biotherapy) than it was in the control group (52%). The G allele's OR was 3.3 (95% CI: 1.58–7.01) in Bio vs. Control and 5.1 (95% CI: 2.31–11.51) in Chem vs. Control.

These results indicate a strong correlation between a higher risk of RA in this population and the C allele and CC genotype of rs17878467.

BIRC5 Expression across Genotypes

Table 8 illustrates Regarding the rs9904341 SNP, the bio-therapy group (1.58 ± 0.0) had a higher expression level of BIRC5 in individuals with the GG genotype than both the chemotherapy group (0.79 ± 0.29) and

the control group (1.14 ± 0.49), although the difference was not statistically significant (P = 0.4). For the GA genotype, the biotherapy group (1.31 ± 0.51) showed a non-significant increase in BIRC5 expression in comparison to the control group (1.35 ± 0.49) and chemotherapy group (0.97 ± 0.0) (P = 0.7). The AA genotype, on the other hand, showed a highly significant difference, with the chemotherapy group exhibiting significantly lower expression (0.55 ± 0.26) than the bio-therapy (0.93 ± 0.27) and control groups (1.00 ± 0.0) (P = 0.001).

There were notable variations in BIRC5 expression between genotypes with respect to the rs17878467 SNP. When compared to the bio-therapy (0.94 ± 0.27) and control groups (0.96 ± 0.04), individuals with the CC genotype in the chemotherapy group exhibited significantly lower expression (0.53 ± 0.23) (P = 0.001). In a similar vein, the biotherapy group's CG genotype expression was significantly higher (1.26 ± 0.52) than that of the chemotherapy group (0.52 ± 0.26) (P = 0.02). For the GG genotype, the bio-therapy group (1.77 ± 0.0) showed a higher expression level than the control group (1.03 ± 0.38) and chemotherapy (0.92 ± 0.06), but the difference was not statistically significant (P = 0.09).

Table 8: Comparison between patients Groups (Chemo therapy and Chem therapy) and control groups in BIRC5 in rs9904341 and rs17878467.

BIRC5		Groups			P-value
		Chemo therapy	Bio therapy	Control	
		Mean ± SD	Mean ± SD	Mean ± SD	
rs9904341	**GG**	0.79 ±0.29	1.58 ±0.0 A	1.14 ±0.49	0.4 NS
	GA	0.97 ±0.0	1.31 ±0.51 A	1.35 ±0.49	0.7 NS
	AA	0.55 ±0.26 b	0.93 ± 0.27 a B	1.00 ±0.0 a	0.001**
rs17878467	**CC**	0.53 ±0.23 b	0.94 ±0.27 a B	0.96 ±0.04 a	0.001**
	CG	0.52 ±0.26 b	1.26 ±0.52 a A	1.41 ±0.44 a	0.02*
	GG	0.92 ±0.06	1.77 ±0.0 A	1.03 ±0.38	0.09 NS

Data were expressed as mean ± SD. Statistical analyses were performed by ANOVA followed by Post Hoc test (Duncan's test) for multiple comparisons. ANOVA significance test (2-tailed). Significant values are bolded. Different small letters indicate significant differences in the horizontal comparisons, whereas different capital letters indicate significant differences in the vertical comparisons. Means followed by the same letter are not significantly different. SD: Standard Deviation, NS: no significant difference. *: Significant (P≤0.05). **: Highly Significant (P≤0.01).

Multivariate Logistic Regression Model for Predicting RA

The independent predictive value of BIRC5 gene expression and SNP genotypes (rs9904341 and rs17878467) for RA susceptibility was assessed using multivariate logistic regression analysis. The model indicated that the rs9904341 GG genotype had a highly significant protective effect against RA (OR = 0.0036, 95% CI: 0.00025–0.053, p = 0.00004), and that the GA genotype of the same SNP had a similar protective effect (OR = 0.024, 95% CI: 0.002–0.255, p = 0.0019). Both genotypes demonstrated strong associations with decreased disease risk, as indicated in Table 9. A good model fit was indicated by the model's intercept, which was likewise statistically significant (p = 0.00005).

Table 9. Multivariate Logistic Regression Model for Predicting Rheumatoid Arthritis Based on BIRC5 Expression and SNP Genotypes.

Predictor	Odds Ratio (OR)	95% Confidence Interval	p-value
Intercept	120.80	11.81 – 1235.33	0.00005
rs9904341 (GA)	0.024	0.002 – 0.255	0.0019
rs9904341 (GG)	0.0036	0.00025 – 0.053	0.00004
rs17878467 (CG)	1.66	0.34 – 8.14	0.530
rs17878467 (GG)	0.87	0.063 – 11.87	0.915
BIRC5 expression	0.347	0.091 – 1.329	0.122

However, in the multivariate setting, none of the genotypes for rs17878467 demonstrated a significant contribution. While the GG genotype demonstrated a non-significant association (OR = 0.87, 95% CI:

0.063–11.87, p = 0.915), the CG genotype displayed an OR of 1.66 (95% CI: 0.34–8.14, p = 0.530). In the adjusted model, there was no significant correlation between BIRC5 gene expression and RA (OR = 0.347, 95% CI: 0.091–1.329, p = 0.122). This suggests that when SNP genotypes are taken into account at the same time, gene expression might not be a reliable independent predictor.
With the GG genotype exhibiting the strongest protective association, these results imply that the rs9904341 polymorphism in the BIRC5 gene may function as a significant genetic marker for RA susceptibility in the Iraqi population under study.

Discussion

Demographic and Clinical Characteristics

The average age of the participants was a little less in the control condition (46.17 +- 13.52) than in Chemotherapy (49.11 +- 9.32) or Biotherapy (45.94 +- 9.85) condition, but the difference did not have any statistical significance (p = 0.4). This indicates that age will not be a major confounding factor in this study population because the three groups are age-matched to a great extent.
Age matching has been used in many studies on autoimmune and rheumatoid arthritis to maintain comparability of the patients and the healthy controls (9).
There was no significant difference in the values of BMI among the groups (Chemotherapy: 28.66 +- 5.45; Biotherapy: 28.71 +- 4.42; Control: 26.59 +- 3.11). Although BMI was marginally greater among patients, this was not significant, which suggests that BMI is unlikely to mediate immune or inflammatory markers interpretation among this cohort.
A meta-analysis of Choy et al. reported the relevance of BMI adjustment in biomarkers of inflammatory diseases, but this effect size is usually very small (10).
There was a very noticeable difference in Sex distribution (p = 0.006). Control group was balanced in terms of male to female (50% each) and chemotherapy and biotherapy had mostly female patients (86% and 74% respectively). This is in line with higher occurrence of autoimmune and rheumatic diseases, which is well established in females.
As an example, rheumatoid arthritis and autoimmune diseases, which affect the system, occur three times as often in women (11).
Both treatment groups (Chem: 33.4 +- 10.4; Bio: 32.85 +- 11.36) had very large differences between them and controls (12.00 +- 4.24), with very significant differences (p = 0.001). This is an indication of persistent inflammation or disease activity amongst the patient groups, even under treatment.
High ESR is a characteristic of persistent inflammation and has been confirmed to be an alternative marker of disease activity in diseases like rheumatoid arthritis and lupus (12).
RF positivity was significantly higher (p = 0.001) with the most positivity observed in the biotherapy group, then in the chemotherapy group (57 and 43% respectively) and lastly the controls (13%). These findings highlight the usefulness of RF as a diagnostic and prognostic biomarker in autoimmune conditions and patients taking biologics who may be more severe or seropositive phenotypes of diseases.
RF positivity has previously been found to predict the severity of disease and poor prognosis in rheumatoid arthritis and is frequently utilized in directing the approaches of treatment (13).
The remarkable increase in the presence of C-reactive proteins in patients with rheumatoid arthritis and more so those receiving biologic pharmacotherapy supports the existence of the inflammatory milieu that accompanies the presence of the disease. Higher CRP is a strictly proven biomarker of general inflammation, and is commonly utilized clinically in gauging treatment reaction and examining unit of disease process in rheumatoid arthritis. Our findings are in agreement with the results of previous studies that have reported an increase in the levels of CRP in cohorts of rheumatoid arthritis compared to healthy control groups (14).
In general, Sex, ESR, RF and CRP status were significantly different, which highlights their diagnostic and clinical importance in the patients of autoimmune diseases in comparison with the controls. The BMI and age did not differ considerably, which is expected to imply an adequate matching and minimized bias.

Expression Levels of BIRC5 mRNA

The results of Table 3 indicate that there is a strong tendency: the relative expression of BIRC5 gene (survivin) is much greater in the RA patient group under the influence of chemotherapy than in healthy controls (fold change 0.411; p = 0.001). Even though the biologic-therapy subgroup demonstrates an intermediate value (0.97-fold) that is not statistically different in comparison to controls, the general

increment in the chemotherapy arm indicates that survivin up-regulation can be related to active disease conditions or inefficient disease-modifying therapy. This result correlates with the immunomodulatory effect of BIRC5 that has been mentioned in the autoimmune conditions such as RA and SLE before (15). Survivin has also come to be identified as a major regulator of autoimmune joint disease. Specifically, Erlandsson et al. established that sustainment levels of survivin in CD4+ T cells mediate a glycolytic transition and promotes invasive phenotypes in synovial fibroblasts of the RA patients (16). This mechanistic observation is consistent with our observation of high-levels of BIRC5 mRNA in patients: persistence of pro-inflammatory, apoptosis-resistant cells in the synovium may be indicated by survivin up-regulation. This outcome is similar to that report, and it presents new data in an Iraqi cohort (17).

Moreover, the latest epigenetic literature on BIRC5 indicates that post-transcriptional control through miRNAs can change the gene expression in RA. Indicatively, Al-Yahya et al. and demonstrated that BIRC5 is controlled by non-coding RNAs in inflammatory environments, and high expression is associated with the lack of therapeutic response in certain cases (18). In this regard, the finding of a major increase in the level of expression in the chemotherapy group can suggest that patients that are poorly adapted to the therapy continue to express survivin-based pathways that maintain the disease.

A curious twist of our data is that even biologic-therapy group with his sophisticated treatments experienced no significantly reduced expression as compared to controls (fold 0.97, $p > 0.05$). This brings two possibilities which include the survivalin expression having been normalized in a subgroup of patients undergoing the biologic therapy or the cohort may consist of patients who have survived with residual active disease despite therapy. It insinuates that survivin expression as an individual might not be sufficient to differentiate completely treated patients. This observation is associated with the perception that survivin is not necessarily a diagnostic marker, but a treatment-refractive disease or disease activity marker (19).

The strength of our study methodologically is that, the comparative Ct method ($2^{-\Delta\Delta Ct}$) was used in quantifying the mRNA so that the calculation of fold-change is reliable (20). We normalized the GAPDH to be the internal reference and demonstrated strong differences in expression by comparing the DCt values of the groups (21).

In short, the results of Table 3 tend to justify the hypothesis according to which survivin up-regulation is linked to the presence of RA and worse reactivity to conventional DMARDs. High levels of BIRC5 can be used as a biomarker of disease activity or resistance to treatment and not as a diagnostic one. The research should focus on the dynamics of this in the future and determine whether it can be used in personalized treatment strategies.

3. Genotypic Distribution and Hardy-Weinberg Equilibrium

In the current study, the detailed evaluation of the genotypic distribution of the two BIRC5 polymorphisms was done, namely, rs9904341 and rs17878467 within the framework of three separate Cohorts: control group, chemotherapy group, and biotherapy group. Our acquired data indicate that we have significant differences in the allele and genotype frequencies, the most significant of which is observed in chemotherapy cohort.

rs9904341 Genotypic Distribution

Genotypic distribution of the mutation rs9904341 showed that there was a strong preponderance of homozygous mutant genotype AA in the chemotherapy group (91.4) but the GG genotype was dominant in the controls (50) and the bio-therapy subgroup (2.9). This dramatic change in the genotype frequencies of patients who have undergone conventional chemotherapeutic agents would put this single nucleotide polymorphism into the category of controlling therapeutic response or susceptivity (22). Importantly, this polymorphism GA type genotype showed statistically significant negative correlation with rheumatoid arthritis when compared to chemotherapy recipients with controls ($p = 0.037$). The mentioned finding highlights the possible significance of heterozygous carriers when it comes to the susceptibility to the disease. Moreover, the G allele was found in the patient group with much higher frequency compared to controls ($p=0.041$) and therefore it is possible that the said allele is a disease-modifying allele in this group of Iraqi population. Recent meta-analytic data support the applicability of the functional relevance of rs9904341 in disease phenotypes: e.g., Malviya et al. (2025) found a consistent relationship between the C-allele of the rs9904341 and high risk in a large-scale study (23). In a similar manner, both the variants, namely, rs9904341 and rs17878467, have been shown by Jimenez et al. (2025) to significantly affect susceptibility in a Latin American population, which explains why these loci are cross-population relevant (24).

Even though the assessment of HardyWeinberg Equilibrium (HWE) did not indicate a significant deviation in the analysis of each of the studied groups ($p > 0.05$), the low percentage of heterozygous carriers of GA (2.9 percentage) in the chemotherapy arm is likely to indicate some possible selective pressures or sample-size restrictions.

These results are in agreement with other studies that suggest that the polymorphism at the BIRC5 promoter region, rs9904341, could potentially regulate the survivin expression and alter the progression of cancer. Indicatively, there was a study that indicated the AA genotype was correlated with increased survivin expression and poorer prognosis of lung and colorectal carcinomas (23). Similarly, Gao et al. (2021) reported that the genotype of the rs9904341-AA was associated with resistance to the application of platinum-based chemotherapy in gastric cancer (25).

rs17878467 Genotypic Distribution

The case of rs17878467 showed that the CC genotype is mostly found in the chemotherapy group (77.1%), and a more equal proportion between the CC, CG, and GG genotypes was recorded in the control group and the biologic-therapy group. Only the chemotherapy group exhibited significant deviation, which is why the Hardy-Weinberg equilibrium test showed significant deviation ($p= 0.01$), which may be a manifestation of a genetic drift, inbreeding, or a significant effect of therapeutic intervention. This is also a breakage of equilibrium, which can mean that the CC genotype is linked to higher incidence of cancer or chemoresistance. It was recently found that the intronic region of BIRC5, marked by rs17878467, could affect the mRNA stability or alternative splicing, and thus changed the expression of survivin isoforms, among which survivin isoforms have been associated with chemoresistance and apoptotic evasion (26)(27). On the other hand, the biologictherapy cohort had more genotype frequencies of both SNPs that were more in line with HardyWeinberg equilibrium which shows that immune-modulatory interventions might have a less direct selective effect on survivin-associated genetic variants than on cytotoxic therapies. This finding is consistent with the recent works proposing that anti-TNF treatment of rheumatoid arthritis regulates the levels of survivin, thus having the potential to redefine the selective landscape of the BIRC5 polymorphisms (28).

Genotype and Allelic Associations

As demonstrated in Table 6 and Table 7, there are deep variations in the genotype and allele composition of BIRC5 gene polymorphism of the BIRC5 genes (rs9904341 and rs17878467) in patients with rheumatoid arthritis (RA) and healthy subjects. In the case of the rs9904341, the AA type was significantly over-represented in patients (e.g., 91.4 per cent in chemotherapy group versus 3 per cent in the control group; OR = 32.2, $p = 0.0001$), and the A allele also showed a significant correlation with the disease status. This dramatic enrichment implies that rs9904341 could be a driver variant affecting RA susceptibility/progression. This SNP has also been reported to have a strong effect on survivin expression and risk of disease, especially in Asian cohorts, as observed by other studies in oncologic settings in the past (29). Furthermore, another variant that might be of interest in this Iraqi population was polymorphism rs17878467 associated with a superiority of the CC genotype in patients (77.2^{-} 20 in controls; OR = 47.2, $p = 0.0001$).

These findings are consistent with the fact that prompts that Ter polymorphisms of BIRC5 can influence transcriptional activities or mRNA stability of survivin, which in turn can modulate the apoptotic resistance and immune cell survival. According to a meta-analysis of BIRC5 polymorphisms, it was significantly reported that, in a range of cancers, BIRC5 polymorphism (rs9904341) is a risk factor (e.g. OR = 1.4 C vs G) (25). Most of that work was cancer-focused but the mechanistic extrapolation, the prevention of apoptosis by increased survivin does have a role in autoimmune diseases including RA where hyperplasia of synovium and autoreactive cells in the joint is a feature.

Notably, there could also be some inherent interactions between genetic background and therapy modality in the strong genotype associations that we have seen in our therapy groups. The highest ORs were observed in the patients under chemotherapy, which could be due to the fact that the conventional DMARD exposure can select the cells with the variant alleles- or that the alleles are the cause of poor response to treatment and therefore a higher proportion in the subgroup. Although research on RA in particular is scarce, a previous study found that treatment response to methotrexate had a marginal correlation with survivin polymorphism (31G -31C) ($p = 0.046$) (7). Although this form is not similar to ours, it indicates the idea of BIRC5 SNPs as a pharmacogenetic activity in RA.

The allele-disease associations of the current cohort have very large ORs, indicating large effect sizes, but one should take this with a grain of salt consideration of the possible limitation in sample size, stratification of the population and bias induced by therapy. Such findings should be confirmed in independent Iraqi and Middle Eastern cohorts in the future, as well as on gene -treatment interactions and functional assays of genotype-independent survivin gene expression.

BIRC5 Expression across Genotypes

A comparison of the BIRC5 mRNA expression on genotypes of the SNPs rs9904341 and rs17878467 is detailed in table 8 in three genotypes; chemotherapy, biotherapy and control. The results indicate that BIRC5 expression depends on the genotype and the situation of the treatment.

rs9904341

In the case of rs9904341 as presented in figure 5, the genotype-based difference in survivin level was significantly important (P = 0.001) between the AA and the CC genotypes. The lowest level was chemotherapy (0.55 ± 0.26), the intermediate was biotherapy (0.93 ± 0.27), and the highest was control (1.00 ± 0.00). It means that the expression of BIRC5 in AA genotype carriers during chemotherapy is strongly reduced. Comparatively, both carriers with GG or GA genotypes had no significant expression differences between groups (P = 0.4 and P = 0.7 respectively). In this way, AA genotype seems to be highly sensitive to BIRC5 downregulation by treatment.

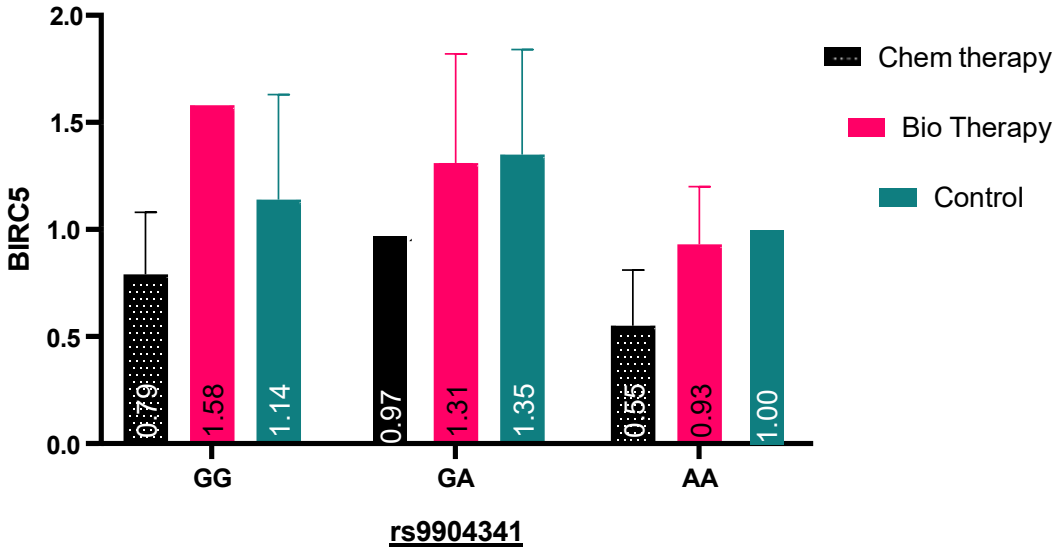


Figure 5: Comparison between patients Groups (Chemo therapy and Chem therapy) and control groups in BIRC5 in rs9904341.

This trend is consistent with larger evidence that BIRC5 promoter region polymorphisms have the capacity to regulate treatment stress gene expression. Indicatively, Siragusa et al. (2024) conducted a literature review on the finding that chemoresistance is involved in survivin overexpression and that regulatory variants can suppress this expression in cytotoxic therapy (30).

rs17878467

Similarly, rs17878467, as illustrated in figure 6, showed significant differences in expression for the genotypes of CG (P = 0.02) and CC (P = 0.001). The control group had the highest CC expression (0.96 ± 0.04), whereas the chemotherapy group had the lowest (0.53 ± 0.23). The expression of CG was higher in the biotherapy and control groups than in the chemotherapy group (1.26 ± 0.52 vs. 0.52 ± 0.26). Statistical significance was not attained by the GG genotype (P = 0.09). These results imply that the response of CC and CG carriers to treatment type differs in terms of survivin expression.

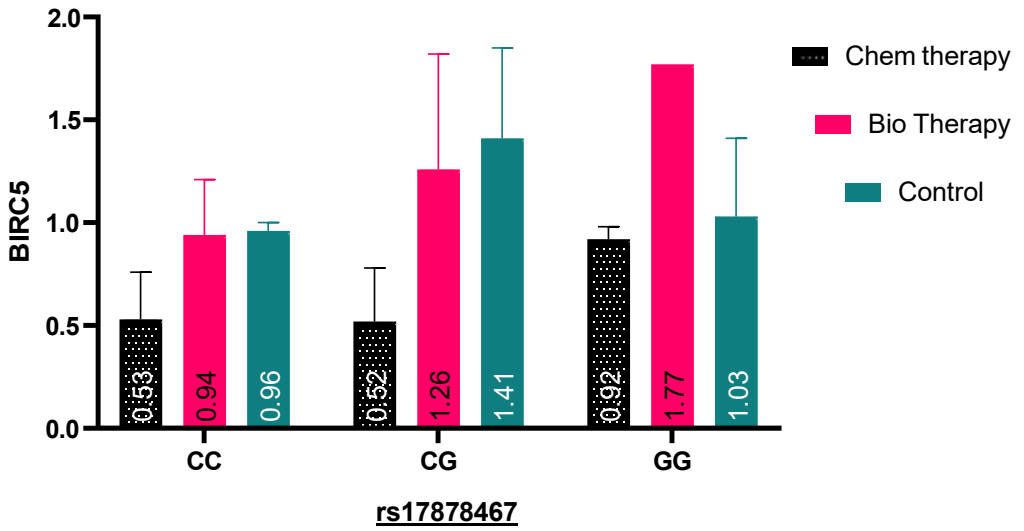


Figure 6: Comparison between patients Groups (Chemo therapy and Chem therapy) and control groups in BIRC5 in rs17878467.

Accordingly, rs17878467 (among other BIRC5 SNPs) correlates with altered gene expression and cancer susceptibility in a Mexican cohort, according to a study by Mumlek et al. (2023) (6). The mechanistic implication is pertinent even though it is in a different disease context: SNP variants in BIRC5 may change transcriptional regulation or mRNA stability, which could impact the treatment's capacity to modulate expression.

The observed patterns of differential expression indicate that treatment type and genotype interact to modulate BIRC5 expression. In particular, in some genotyped carriers (AA for rs9904341; CC for rs17878467), chemotherapy seems to be more successful in lowering survivin expression, whereas biotherapy and control groups continue to exhibit higher expression. This could be because biologic therapies might not put as much strain on survivin transcription or might use different survival strategies, while cytotoxic treatments directly inhibit survivin-mediated anti-apoptotic pathways. The BIRC5 SNP genotype is therefore positioned as a potential biomarker for treatment stratification, as carriers of specific genotypes may benefit more from particular types of therapy.

Multivariate Logistic Regression Model for Predicting RA

The purpose of the multivariate logistic regression model presented in Table 8 was to evaluate the predictive significance of two SNPs, rs9904341 and rs17878467, and BIRC5 expression on the risk of rheumatoid arthritis (RA). In order to find independent predictors, this thorough model accounted for the combined impact of gene expression and genotypic variations.

rs9904341 Association with RA Risk

There was a noteworthy correlation between the rs9904341 polymorphism and RA susceptibility. In particular, the GG genotype showed a significant protective effect (OR = 0.0036; 95% CI: 0.00025–0.053; p = 0.00004) and the GA genotype showed a significantly lower odds ratio (OR = 0.024; 95% CI: 0.002–0.255; p = 0.0019). These results imply that the G allele plays a significant protective role against RA. This is consistent with research by Zhou et al. (2019), who found that the rs9904341 G allele in the BIRC5 promoter may limit the anti-apoptotic activity linked to chronic inflammation in autoimmune diseases by lowering survivin expression (23). Reduced expression of survivin may lessen joint damage and the progression of autoimmunity because of its function in synovial cell proliferation and resistance to apoptosis.

rs17878467 Association with RA Risk

Neither the GG genotype (OR = 0.87; p = 0.915) nor the CG genotype (OR = 1.66; p = 0.530) demonstrated a statistically significant association with RA risk for rs17878467. Despite showing a slightly higher OR, the CG genotype may not be a reliable or independent predictor, as indicated by the high p-values and wide confidence interval.

Although little is known about the function of rs17878467 in autoimmune diseases, MM Abdulkarim's study from 2023 found no evidence of a significant correlation between this variant and susceptibility to systemic autoimmune diseases, which is in line with our findings (31).

BIRC5 mRNA Expression as a Predictor

Remarkably, the logistic regression model did not show BIRC5 gene expression as a statistically significant independent predictor (OR = 0.347; 95% CI: 0.091–1.329; p = 0.122). Although earlier univariate analyses revealed that RA patients had higher levels of survivin expression, the predictive power of this analysis decreased when genotypes were added to the model. This implies that either these SNPs mediate expression differences or that genotypic variations in BIRC5 may have a more significant effect on RA susceptibility than mRNA expression alone (32).

Variable survivin expression in RA has been shown in studies like Zafari et al. (2019) based on treatment status and disease stage, suggesting that expression levels may not be a reliable biomarker across patient populations (33).

Conclusion

This paper presents strong evidence to show that the BIRC5 (Survivin) gene, the polymorphisms of which are likely to predispose Iraqi patients to rheumatoid arthritis (RA) and determine the patient-response to disease-modifying antirheumatic drugs (DMARDs), is likely to be the cause of this issue. In particular, the GG genotypes at both loci showed drastically high frequencies in RA patients as compared to the healthy controls, indicating that there might be a risk association. Furthermore, Survivin overexpression seems to play a role in pathogenesis of the disease as well as resistance to conventional therapy as RNA expression studies revealed significantly higher BIRC5 mRNA concentrations in RA patients and especially those receiving biological therapy.

The observed genetic types and allelic patterns, and also the non-observance of Hardy-Weinberg equilibrium in some groups, are also the effects of genetic selection dynamics as dictated by the therapeutic modalities. The results obtained indicate that transcriptomic profiling and genetic screening should be introduced into the clinical evaluation of RA to come up with more personalized and effective treatment plans.

Further multicentered studies that are larger in cohort size and scope of populations are needed to support these associations and to explain the mechanistic roles of BIRC5 polymorphisms and expression in RA immunopathology.

Recommendation

1- Since it was demonstrated that the rabirc5 single-nucleotide polymorphisms share both susceptibility to rheumatoid arthritis and response to different types of therapy, it would be wise to consider genotyping of these variants in future treatment regimes. These integrations would enable identifying patients with a genetic predisposition susceptible to specific disease-modifying agents or those that would respond positively to certain disease-modifying agents.

2- The up-regulation of Survivin messenger RNA, as indicated by the results, and its association with clinical outcomes indicates the possible benefit of using BIRC5 expression as a prognostic biomarker. The inclusion of quantitative expression analysis would help in stratifying the patients especially in patients who may respond to biologic agent use, as compared to the non-biologic conventional disease-modifying anti-rheumatic drugs.

3- Further functional studies, both in cell culture and in vivo, should be done to specify the mechanistic pathways by which these polymorphisms affect Survivin expression and, by extension, immune modulation in the rheumatoid arthritis pathology.

4- To validate the strength of these results and further increase their external validity, research involving the involvement of more patient groups of various geographical locations in Iraq, including its neighboring populations, needs to be conducted. This will reduce the effects of genetic heterogeneity and discover possible environmental interactions, which can modify disease expression.

5- Future research directions need to be searching to find additive polymorphisms in BIRC5 gene, assessing the interaction of combinatoric variations on disease risks and treatment patterns, possibly by undertaking a complete haplotype scanning, or genome-wide association analysis.

6- Combining the expression measures of Survivin and SNP genotyping with the already validated rheumatoid arthritis biomarkers anti-cyclic citrullinated peptide antibodies, rheumatoid factor values, and cytokine profiles might improve the accuracy of disease diagnosis and support the creation of advanced therapeutic stratification analyses.

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