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Association between Insulin like Growth Factor-1 Gene Polymorphisms (Rs35767) And Progression of Rheumatoid Arthritis in Iraqi Patients

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Abstract. Rheumatoid arthritis (RA) is a prevalent, chronic autoimmune condition that affects the synovial membranes and articular tissues of many joints. Aim: detect the association between rs35767 in the promoter of the *IGF-1* gene with serum level of IGF-1 protein and the development of RA. The study included 120 RA patients, which are divided into two groups (newly diagnosed and treated) in addition to the third group consisted of 60 healthy individuals. Using sandwich ELISA technique to estimate serum level of IGF-1, IGF-1R, and IGFBP3 . designing specific primer to detect the rs35767 by tetra-arm technique. GG represented the dominant genotype in patients (47.5%) , 35% ,17.55 for AG and AA respectively .Results revealed that serum level of IGF-1,IGF-1R and IGFBP3 were present at higher levels in newly diagnosed patients than in patients receiving treatment, both groups being higher by a clear and significant amount when compared to healthy subjects .Conclusion rs35677 found to be more common in patients , and can play an important role in the genetic vulnerability to RA diseases

Keywords: Rheumatoid arthritis, rs35767, insulin like growth factor-1.

1. Introduction:

Rheumatoid arthritis is an autoimmune disease that results in chronic inflammation. As a result, the immune system assaults the cells that line joints, which results in pain, swelling, and stiffness. Despite the tremendous advancements in treatment options brought about by new kinds of drugs, severe rheumatoid arthritis can still lead to physical limitations (Testa *et al.*, 2021, Al-Rahim *et* al., 2023). Genetic and environmental factors interact to cause RA. Some individuals have a higher risk of developing RA due to a number of inherited factors. RA patients have been found to have an elevated prevalence of more than 100 genetic alterations. Each gene marginally increases the likelihood of acquiring the disease. The genes involved appear to vary between individuals and between populations in different parts of the world (Guo e*t al.*, 2018; van Delft and Huizinga, 2020).

IGF is a circulating peptide hormone that is mostly made by the liver under the control of GH, which is released by the pituitary gland. IGFs essential for an organism's healthy metabolism, growth, and homeostasis. The human IGF-1 gene, which has six exons and produces three primarily heterogeneous transcripts, is found in the long arm of chromosome 12 (Skarlis *et al*.,2019). To exercise all of its known physiological effects, it binds to the IGF-1R, which is controlled by a number of IGF-binding proteins (IGFBPs). To transport up to 75 to 90% of the circulating IGF-1 and control its activity, IGFBP-3 the most significant protein interacts with the IGF-1R receptor In the serum and/or synovial fluid, several rheumatic illnesses, including osteoarthritis, widespread idiopathic skeletal hyperostosis, and RA, have aberrant growth hormone (GH)/IGF-1 levels (Liu et al., 2018; Wen *et al*., 2021; Lee *et al*., 2022).Numerous mechanisms in the IGF-1 pathway can control metabolism in

bones. utilizing a mechanism separate of mitosis, IGF-1 can promote the production and mineralization of the bone matrix and influence bone metabolism by controlling bone cell activities (Frisch *et al*., 2016). IGF-1 is not only encourages the growth and transformation of stromal cells in the bone marrow into osteoblasts, but it also increases the production of bone collagen, Additionally, IGF-1 has the power to stop collagen from degrading by preventing cell death, which promotes the formation of bone matrix (Xian *et al.*, 2012). IGF-1 performs essential functions in osteogenesis, repairing of boner, and the bone regeneration when taken as a whole (Feng *et al.*, 2015; Zidan *et al.*, 2016).rs35767 which have been linked to a number of human disorders, RA and cancer development. It effects the serum level of IGF-1 protein specially in patients in comparison with control (Dhaunsi *et al.*, 2012 ,El-Magd *et al*., 2017 , Qin *et al*.,2019).

Methods:

Patients

The World Health Organization's criteria were used to diagnose a group of 120 RA patients, who were then divided into two groups. The first group consists of patients who are currently receiving treatment (n=60), Patients in the second group who did not receive treatment range in addition to healthy control subjects (n = 60). A standardized questionnaire was used to gather information on the RA risk variables of gender, age, smoking, family history, and others. The study's protocol was approved by the ethics committee at the Al-Yarmouk Teaching Hospital in Baghdad, which is part of the Iraqi Ministry of Health. Each participant in the study gave their written agreement in a signed document.

Five ml of blood drawn using a single-use syringe by venipuncture to obtain blood samples. 2 ml was transferred into an EDTA tube for the necessary hematological analysis and DNA extraction, while the remaining 3 ml was placed into disposable gel tubes and left to stand at room temperature (20–25 °C) for clotting. The serum was separated using centrifugation for 5 minutes at 3000 rpm. serum and kept at -20°C until they were used for estimation serum level of IGF-1, IGF-1R and IGFBP3 by sandwich ELISA according instructions of kits (Sunlong/China).

Genomic DNA from all blood samples were extracted by using Wizard genomic DNA purification Kit (Promega /USA). Then DNA samples were kept at -20°C. To genotype rs35767 in the promoter region of *IGF-1* gene, tetra arm PCR was utilized. special primers were created for this purpose by NCBI (https://www.ncbi.nlm.nih.gov) and utilizing the free online primer creation tool accessible at http://primer1.soton.ac.uk/primer1.html, The components of reaction mixture were listed in table (2) and PCR protocol listed in table (3).

Table (1): Primer for detection of (rs35767) in IGF-1 gene

SNP	**Size (bp)**	**Primer**	**Sequence (5-3)**
rs35767	G allele:173	Inner F	TATAATGTACACTTGCCTTTGCCATTGA
	A allele:260	Inner R	AAACAAAGCAGGTTTGTTTGTTTTTTGTT
	Size of two outer primer =378	Outer F	CCTTGAATTTTTTCTTTTTTTTTTTTTACG
		Outer R	ATGTGTCAGTCCCCTGAGAGTCAGGT

Table (2): Components of Master Mix

Component	Volume (µL)
Taq PCR Pre Mix	12.5
Inner Forward primer	0.5
Inner Reverse primer	0.5

Outer Forward primer	0.5
Outer Reverse primer	0.5
DNA	5
Nuclease free water	5.5
Total	25

Table (3): PCR program for detection of rs35767

Stage	Tm (°C)	Time	cycle
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	58	30 sec	
Extension	72	30 sec	
Final Extension	72	5 min	1

The result of the amplification procedure was determined by electrophoresis of the with 2% agarose for 2 hours (70 volts) and using the (100bp) DNA ladder as a size mark for determining the size of the amplified fragment.

SPSS 20.0 was used to conduct the statistical analysis. Using a chi-square test, categorical variables were examined. Continuous normal distribution variables with a mean as well as a standard deviation were examined by samples that were independent ANOVA for between-group comparisons.

Results

The findings of the current study are presented in table (4), which revealed that all cytokines were present at higher levels in newly diagnosed patients than in patients receiving treatment, both groups being higher by a clear and significant amount when compared to healthy subjects

Table (4): Serum Levels of IGF-1, IGF-1R and IGFBP3 in patients group and healthy volunteers

Group	Newly	Treated	Control	P-value
IGF-1 (ng/ml)	157.17a±43.8	73.69b±21.78	39.52c±15.21	0.001
IGF-1R (pg/ml)	5581a±1105	3742.8b±553.8	2659.4c±680.4	0.001
IGFBP3 (ng/ml)	132.31a±16.98	100.98b±22.32	65.8c±11.77	0.003

Hardy-Weinberg equilibrium (HWE) demonstrated that the three genotypes of rs35767 were deviated significantly from HWE in rheumatoid arthritis patients and non-deviated in healthy control, as no significant variations were found between observed and expected frequencies table (5).

Table (5): Hardy-Weinberg equilibrium analysis of IGF-1 rs35767 polymorphism in RA patients and healthy control.

Genotypes	Patients (N = 120)				Control (N = 60)			
	Observed		Expected		Observed		Expected	
	N	%	N	%	N	%	N	%

AA	21	17.5	14.7	12.3	3	5.0	1.5	2.5
AG	42	35.0	54.6	45.5	13	21.7	16.0	26.7
GG	57	47.5	50.7	42.2	44	73.3	42.5	70.8
HWE-*p*-value	0.012				0.147			

GG represented the dominant genotype in patients (47.5%), 35% 17.55 for AG and AA respectively Gene polymorphisms were found to be more common in patients than in healthy controls, and can play an important role in the genetic vulnerability to RA diseases. It is caused change levels of the IGF-1, which is involved in the development risk of RA diseases. Distribution rs35767 genotypes of the *IGF-1* gene differed significantly between patients with RA and controls The ratio of patients A allele 84 %, allele G 156 %, while the frequency of healthy A allele 19 %, allele G 101 %.. The allele G is more common in patients compared to control.

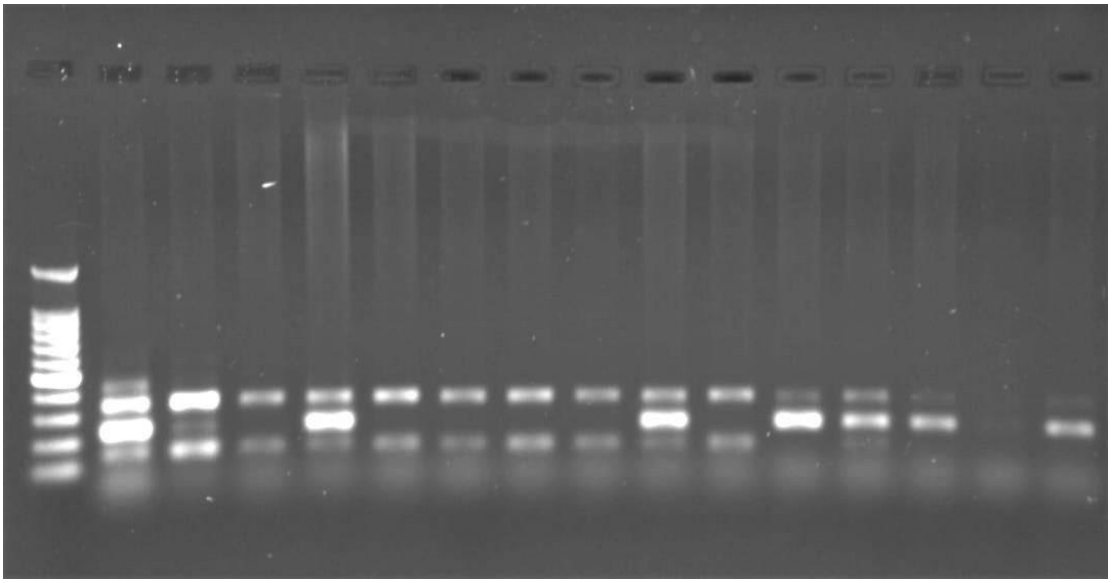

Figure (1): Genotypes for rs35767 of IGF-1 gene in rheumatoid arthritis patients and control.

Distribution rs35767 genotypes of the *IGF-1* gene differed significantly between patients with RA and controls, The frequency of genotype AA was particularly increased in patients than control (17.5 *vs*. 5.0 %; p = 0.020) Such deviation scored an OR value of 4.03 (95% CI 1.16 – 13.97). In the patients the frequency of genotype AG showed a significantly increased than the control (35.0 *vs*. 21.5 %; p =0.086; OR = 1.95; 95%CI = 0.95 - 3.98), and the frequency of genotype GG in control (73.3 %) was significantly increased compared to patients (47.5 %) with (p = 0.001; OR =0.33; 95%CI = 0.17 -0.64)

Table (6): Logistic regression analysis of *IGF-1* polymorphism in RA patients and control.

Allele/ Genotype	Cases (N = 120)		HC (N = 60)		OR	95% CI	*ρ*-value
	N	%	N	%			
A	84	35.0	19	15.8	2.86	1.64 - 4.99	< 0.001
G	156	65.0	101	84.2	0.35	0.20 - 0.61	< 0.001
AA	21	17.5	3	5.0	4.03	1.16 - 13.97	0.020
AG	42	35.0	13	21.7	1.95	0.95 - 3.98	0.086

GG	57	47.5	44	73.3	0.33	0.17 - 0.64	0.001

Among RA patients, the three genotypes significant influence serum level of IGF-1, AA genotype of rs35767 AA gave a strong association between serum levels of IGF-1 and disease progression. Serum levels of IGF-1 were higher in various types of RA patients, correlating with disease severity.

Table (7): Association between genotypes of rs35767 and serum level of IGF-1 among investigated groups

Genotypes	**Serum level of IGF-1**		
	Newly (60)	**Treated(60)**	**Control(60)**
AA	205.56^{a}±23.69	100.83^{a}±10.93	60.25^{a}±13.23
AG	190.41^{a}±23.25	80.36^{b}±10.34	52.84^{a}±11.18
GG	124.39^{b}±26.04	52.86^{c}±15.4	34.17^{b}±12.71
P-value	<0.001	0.001	0.003

4. DISCUSSION

RA is a chronic inflammatory disease that affects the entire body with no known cause. Leucocyte infiltration, immunological activation, and untimely synovial inflammation that results in joint swelling are its most significant characteristics. (Smolen et al., 2016).

It takes an enormous structure of interacting cells and cytokines to control immunity and inflammation. Growth hormones, especially insulin-like growth factor-1 (IGF-1), have a variety of roles in the regulation of the immune system, which is controlled by the endocrine system, IGF-1 has a role in regulating immunity and inflammation as well as promoting bone and cartilage tissue development and differentiation (Matsumoto and Tsurumoto, 2002).

IGF-I levels in the RA patients' plasma and SF were shown to be higher than those in controls in several investigations during the past few years (Matsumoto and Tsurumoto, 2002, Smolen et al., 2016), IGFBPs and IGF-1 have also been linked to the presence of proinflammatory cytokines such interleukin-1 beta (IL-1), tumor necrosis factor-alpha (TNF), and C-reactive protein (CRP). (Neidel, 2001). In comparison to healthy controls, RA patients had higher blood levels of IGF-1. In the RA synovium, macrophages were predominantly responsible for IGF-I synthesis, these findings suggest that inappropriate osteoplastic activation and angiogenesis in RA are related to aberrant IGF-I production (Suzuki *et al*., 2015).T cell-dependent inflammation in arthritic situations is influenced by IGF-1R signaling. By lessening arthritis discomfort and preventing the expansion of IL6-dependent Th17 cells (Erlandsson *et al*., 2017).

The current study's findings demonstrated that there were observable, substantial changes in IGFBP3 levels between patients and healthy people.IGF-1 is a possible target since it affects how inflammatory illnesses start. IGFBP amount and isoform, which are important signaling variables, have an impact on IGF availability for receptor binding (Lee et al., 2022). In the present study, we examined the genotype of the SNP rs35767 from the promoter region of IGF-1 in RA patients. In the study, we showed that carriers of the AA genotype may be more likely to develop RA than carriers of the GG genotype. Under the dominant hypothesis, bearers of the AG and AA genotypes may be more likely to develop RA (OR = 4.03, 95% CI 1.16 - 13.97, P = 0.020). A substantial correlation between the frequency distribution of the A allele and illness risk was found (P = 0.001). These results might present an original approach for RA screening. According to rs35767, the SNPs may be significant in controlling IGF-1 levels (Chen et al., 2017). This study focused on the SNP polymorphism rs35767 in the IGF-1 promoter region. Another study (Zhang et al., 2017). looked into the variants of the IGF-1 gene associated with the likelihood of getting diabetic retinopathy in the Han Chinese population. They

discovered, in agreement with our findings, that the minor allele homozygote of rs35767 (TT) was clearly linked with blood IGF-1 levels.

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