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Evaluation of Serum level and IL 4R (rs1805010) gene polymorphism in a sample of osteoporosis women

Zainb Abdulrahman Hammadi[1], Maan Hasan Salih[2], Firas Faris Rija[3]

[1+2 +3] Department of Biology, College of Science, Tikrit University, Tikrit, Iraq

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***Corresponding author:**
Zainb A. Hammadi
Email :
zainb.a.hammadi.bio2022227@st.tu.edu.iq

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Abstract

Background: Osteoporosis is characterized by subtle skeletal degeneration and bone loss, generally defined as a systemic metabolic disease. Interleukin-4 receptor (IL-4) R has been associated with osteoimmune, the relationship between the immune system and the skeleton (osteoimmune system) is studied in osteoimmunology. **Objectives:** In this study, we aimed to investigate the serum level and polymorphisms to genotype IL-4R) rs1805010) and their effect on osteoporosis in Iraqi women. **Methods:** ELISA was used to determine the serum level of the IL-4R and polymerase chain reaction (PCR) to genotype the IL4R gene in 90 Iraqi women (60 patients with osteoporosis and 30 control groups). **Results:** The main findings on the IL-4R polymorphism (rs1805010) indicated that there was no association for the prediction of osteoporosis in Iraqi women. Whereas, there was a significant increase in serum levels of interleukin-4 receptor in women with osteoporosis compared to healthy women, (P = 0.018). **Conclusion:** The study supposes that there is a link between the serum level of IL4R and how osteoporosis develops, but IL4R gene polymorphisms may not play a role in osteoporosis in women.

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تقييم المستوى المصلي وتعدد الأشكال الجيني لـ IL 4R (rs1805010) في عينة من النساء المصابات بهشاشة العظام

زينب عبد الرحمن حمادي **معن حسن صالح** **فراس فارس رجا**

الخلاصة

الخلفية: هشاشة العظام هو مرض يتسم بانحلال الهيكل العظمي وفقدان العظام، والذي يُعرَّف عمومًا بأنه مرض أيضي جهازي. يرتبط مستقبل إنترلوكين 4 **(IL-4R)** بالمناعة العظمية. **الأهداف:** هدفت هذه الدراسة إلى معرفة مستوى المصل وتعدد الأشكال لـ **IL-4R) rs1805010** (وتأثيرهما على هشاشة العظام لدى النساء العراقيات. **الطريقة:** استخدام تقنية الاليزا لتحديد مستوى المصل من **IL-4R** وتم استخدام تفاعل البلمرة المتسلسل **(PCR)** في التركيب الجيني لجين **IL4R** في 90 امرأة عراقية (60عينة مصابات بهشاشة العظام و30 عينة كمجموعة ضابطة). **النتائج:** النتائج التي توصلنا إليها حول تعدد الأشكال **IL-4R (rs1805010** تشير إلى أنه لا يوجد ارتباط للتنبؤ بهشاشة العظام لدى النساء العراقيات. في حين كانت هناك زيادة معنوية في مستويات مصل مستقبل الانترلوكين 4 في النساء المصابات بهشاشة العظام مقارنة بالنساء الأصحاء، **(P = 0.018**). **الأستنتاج:** نقترح أن هناك ارتباط بين مستويات **IL4R** في الدم والتسبب في مرض هشاشة العظام، في حين أن تعدد الأشكال الجينية **IL4R** قد لا تساهم في هشاشة العظام عند النساء.

Introduction

Osteoporosis (OP) is a medical condition characterised by diminished bone strength, resulting in an elevated susceptibility to fractures (1). The condition impacts a substantial population, encompassing individuals of all genders and ethnic backgrounds, with its frequency projected to rise in tandem with the aging demographic. Osteoporosis remains asymptomatic until the occurrence of fractures, which after that give rise to notable secondary health complications and potentially fatal outcomes (2). The prevalence of OP is steadily increasing and becoming a major public health problem as life expectancy increases faster in developing countries, In postmenopausal Iraqi women the incidence of OP disease increased to 22.8 %(3). This chronic disorder has a prevalence rate of one in three among women and one in five among men aged 50 and above. According to the World Health Organization (WHO), osteoporosis is characterized by a bone density that falls below 2.5 standard deviations (SD) in relation to the average bone density of a healthy population of the same age and sex. The Osteoporosis disease leads to a decline in both bone density and quality, thereby earning the designation of "porous." (4). Osteoporosis is classified as primary, which occurs in postmenopausal women and people over the age of 70, and secondary OP which occurs as a result of other diseases or treatments (5). There is a complicated interaction between genetic, intrinsic, external, and behavioral variables that leads to osteoporosis (6). Osteoporosis occurs as a result of the disruption in the equilibrium between bone resorption, facilitated by osteoclast cells, and bone synthesis, facilitated by osteoblast cells (7) the immune system assumes a prominent role in this disease progression, exerting its influence through immune cells, diverse cytokines, and signalling pathways. The interaction between the skeletal system and the immune system gives rise to a multidisciplinary area of study known as Osteoimmunology (8). The IL4R gene is located on chromosome 16p (16p12.1) (9). The human IL-4 receptor alpha (IL-4Ra) is present in two forms: a membrane-bound receptor known as IL-4R, and a soluble receptor referred to as soluble IL-4Ry. The second kind is made when the membrane-bound form is broken down by proteases (10). The IL-4 receptor

plays a crucial role in mediating the transmission of extracellular IL-4 and IL-13 cytokine signals into distinct cell-specific responses, hence impacting various biological processes, including the immune response. The interleukin-4 receptor (IL-4R) exhibits a significant degree of polymorphism; nevertheless, the functional characterization of these variants remains limited to a select handful (11). Osteoporosis is a highly polygenic and heritable disease, hence, this study was planned to determine if serum level and allelic variants of IL-4R polymorphism (rs1805010) pathway may be used as a target for future Pharmacogenetics of Osteoporosis. The purpose of this study is to evaluate genetic polymorphism and serum levels of IL4R with osteoporosis susceptibility in Iraqi women.

Materials and Methods

Patient and methods: A total of 90 samples were taken from women aged from 26 to 90 years, 60 women with osteoporosis group among a sample of women who visited the Dijlah Rehabilitation Hospital - Tikrit /Iraq, and 30 healthy women group. The calculation of body mass index (BMI) involves the division of weight in kilograms by the square of height in meters (kg/m2) (12). Bone mineral density (BMD) was measured in women with osteoporosis using a DEXA scan (Diagnostic Medical System Version: V3.0.8.313, France). The WHO characterizes osteoporosis based on the following bone density levels: bone density is considered normal if the T-score > -1, osteoporosis if the T-score is -1 to -2.5, and osteoporosis if the T-score <-2.5 (13). After collecting the demographics of the participants, an EDTA anticoagulant tube is used to collect 5 mL of blood samples for biochemical and molecular analysis.

Ethical approval: The Ethics Committee of the College of Science at Tikrit University in Iraq approved the study. Written acceptance was documented by means of a written, signed, and dated informed consent from the patients to participate in this study.

Biochemical Analysis: The Human High Sensitivity (0.8 pg/ml) Interleukin 4 receptor (HS IL-4R) ELISA (SUNLONGBIOTECH, China, Cat NO.:SL3673Hu) was used to measure the serum level of IL-4R in osteoporosis women and healthy women based on ELISA method (Elisa system, Mindray, China).

Molecular analysis: Genomic DNA was extracted from whole blood via a kit procedure (Genaid, Taiwan). The Tetra-ARMS PCR technique was employed to identify the single nucleotide polymorphism (SNPs) of the IL-4R receptor gene. This involved amplifying the specific DNA segments of interest using four custom-designed primers (Table 1) was provided by Macrogen, a Korean company specializing in genetic analysis. The polymerase chain reaction (PCR) was used to amplify Target fragment which contains rs1805010. The detection of PCR products was carried out by the use of gel electrophoresis with a 2% agarose gel. While PCR reactions were carried out using the following program in Table 2.

Table (1): Primers names and their sequences used in the PCR reaction

Primer	Primer sequence (5´ → 3´)	PCR product	Reference
IF10	5´CCTGTGTCTGCAGAGCCCACACGTTTG-3´	G allele- 172 bp A allele- 245 bp Outer bands 364 bp	This study
IR10	5´CCCGCGCCTCCGTTGTTCTCAGGTAT-3		
OF10	5´TAAGAGGCTGTGGCCAGCAAGAGAGGCAA-3´		
OR10	5´GCTCACCATGCTCGCTGGGCTTGAAG-3´		

Table (2): The program used to perform the PCR reaction.

Stage	Temperature	Time	cycles
Initial denaturation	95 C	5 min.	1
Denaturation	95 C	30 sec.	32
Annealing	69 C	45 sec.	
Extension	72 C	45 sec.	
Final extension	72 C	7 min.	1

Statistical analyses: GrapPad Prism 9 was used for the statistical analysis. The allele and genotype frequencies as well as models of inheritances between the osteoporosis and healthy women were analyzed by using the chi-squared test and Fisher's exact test.

Results

In current study, the lowest age group for a total of 60 samples of female patients was 26 years, and the highest age group was 90 years, and their average age (50.52±12.81) years. As for their heights, the least of them was (142 cm) and the long (171 cm), and their average height was (164.7 ± 8.08 cm). The lowest weight value in the 60 samples was (50 kg), and the highest value was (106 kg), and their average weights (81 ± 12.69 kg). The results showed BMI that its lowest value was (18.4 kg/m2) and the highest BMI value (38.1 kg/m2), and it's mean (81 ± 12.69 kg/m2). Three samples appeared with a normal weight, the lowest value of which was (18.4 kg/m2) and the highest value of (24.9 kg/m2), which was the mean (22.2 ± 3.38 kg/m2). Twenty six of the women were overweight the minimum was (23.3 kg/m2) and the maximum (29.8 kg/m2), which was a mean (27.19 ± 1.43 kg/m2). Obesity appeared among 31 samples, with a minimum of (30.1 kg/m2) and a maximum of (38.1 kg/m2), and their mean (33.03 ± 2. 2 kg/m2). See Table 3.

Table 3. Demographic characterization of the patients with osteoporosis

Categories	Number	Minimum	Maximum	Mean ± SD
Age (years)	60	26	90	50.52±12.81
Height (cm)	60	142	171	164.7 ± 8.08
Weight (kg)	60	50	106	81 ± 12.69
BMI (kg/m^2)	60	18.4	38.1	29.96 ± 3.88
normal weight	3	18.4	24.9	22.2 ± 3.38
Overweight	26	23.3	29.8	27.19 ± 1.43
Obesity	31	30.1	38.1	33.03 ± 2. 2

The IL-4R levels in the patients and healthy groups were 33.82 ± 15.86 pg/mL and 19.61 ± 17.54 pg/mL, respectively. The significant differences between the patients' group and control groups (P < 0.018) are clearly apparent in Figure 1.

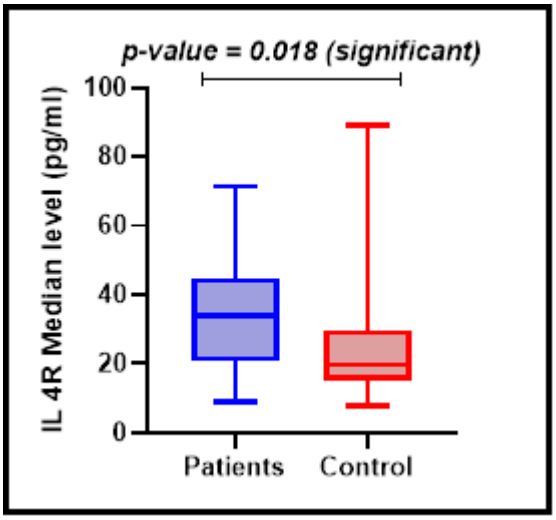

Figure (1): The statistics compared of IL 4 serum levels between patients and the control group:

A study group comprising 60 patients and 30 healthy women underwent genotyping by the process of electrophoretic separation of polymerase chain reaction (PCR) results. Identification of genotyping was successful in 100% of all samples. Two different alleles were detected G- 172 bp and A- 245 bp ; (Figure 4) as well as three genotypes (172/172) (G\G), 172/245 (G\A) and 245/245(A\A) were identified with respect to (rs1805010) of the IL 4R gene in the current study population. Based on the DNA bands, allelic and genotyping frequencies were determined for the measurement of the amount of genetic variation in a study population.

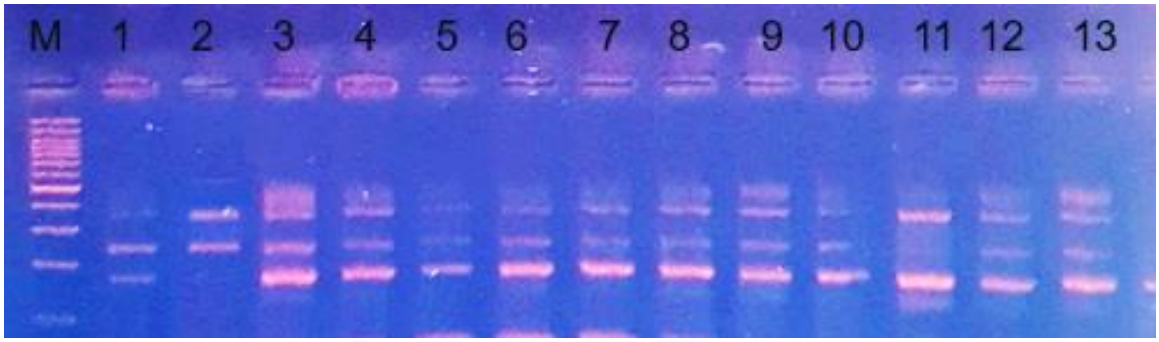

Figure (2): the PCR products of the IL 4R gene are electrophoresis on an agarose gel (2%). M = Marker 100 bp. Well 1, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 13 represent the GA genotype. Well 2 represents genotype AA. Well 11 represents the genotype GG.

The (G) allele was the most common form of an allele and is designated the widespread allele in osteoporosis women **(0.6)** and in healthy women **(0.16).** The second allele (A) frequency was found with **0.4** frequency in cases and with **0.39** frequency in the healthy group. However, allele frequency was not found to be associated with the risk of osteoporosis. The results obtained from the preliminary statistical analysis of allele frequencies are summarized in Table 4.

Table (4): Compares the summary statistics for Allelic distribution of IL 4R in osteoporosis women and healthy women.

Alleles	Study participants								
	osteoporosis No.	Allele frequency in osteoporosis	Healthy control No.	Allele frequency in Healthy control	Allele frequency in study population	OR	95% CI		p-value
							Lower	Upper	
G (reference)	72	0.6	37	0.16	0.822	1			
A	48	0.4	23	0.39	0.188	1.07	0.56	2.02	P = 0.82
Total	120		60						

OR= odd ratio; Cl; confidence interval; $\chi 2$: Chi square.

Three genotypes were identified in the present study. When the GG genotype was considered as the reference group, with 15 and 8 genotypes in both cases and control, respectively. But among three possible genotypes (AA, GA, and GG) for IL-4R gene polymorphism, we only found 3+1 of the AA genotype in both cases and control, respectively. Also, among all models of inheritance, no genotypes were found to be associated with the risk of osteoporosis. The results of genotypic distribution and statistical correlational analysis are set out in Table 5.

Table (5): Genotypic distribution and statistical analyses of IL 4R gene in osteoporosis women and in healthy women.

Genotype	Study population					
	osteoporosis No.	Healthy control No.	OR	95% CI		p-value
				Lower	Upper	
GG (reference)	15	8	1			
GA (RP1\ RP2)	42	21	0.93	0.33	2.63	P = 0.89
AA (RP2\ RP2)	3	1	1.4	0.12	15.97	P = 0.78
Models of inheritances						
Co-dominant inheritance model						
AA vs. GG	3 vs 15	1 vs 7	0.71	0.06	8.15	P = 0.96
Dominant inheritance model						
GG vs GA + AA	15 vs 45	8 vs 22	1.09	0.40	2.95	P = 0.74
Recessive inheritance model						
GG + GA vs AA	57vs 3	29 vs 1	1.52	0.15	15.32	P = 0.56
Over-dominant inheritance model						
GG + AA vs GA	18 vs 42	9 vs 21	1	0.38	2.6	P = 0.74

OR= odd ratio; Cl; confidence interval; χ2: Chi square.

The results of the study shown in (Figure 3) showed that there was no significant difference in the serum Ll-4R level according to the genotypes of the IL-4R gene in both groups of patients and healthy group, and the p-value was equal (0.7), (0.48) to patients and healthy group, respectively.

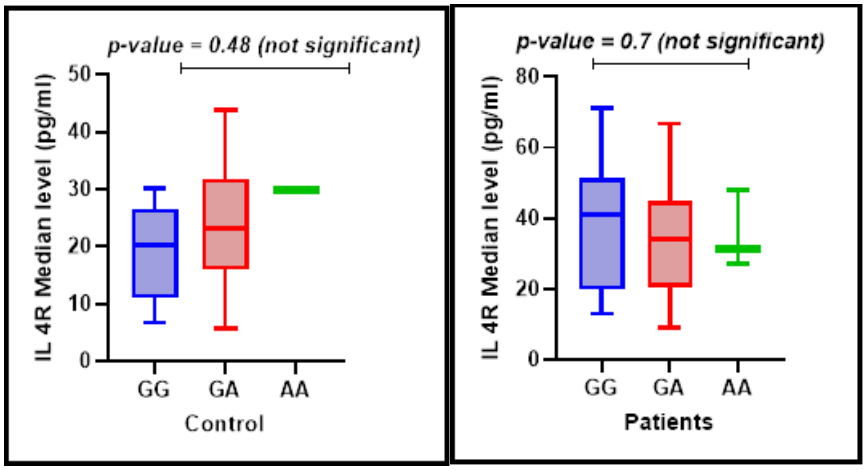

Figure (3): Serum level of IL 4R between the patients and healthy groups according to the genotypes of the IL 4R gene.

Discussion

Interleukin-4 is an important immune cytokine that regulates bone homeostasis in healthy women group (14). The IL-4Rα subunit, which consists of two different subunits, has the ability to bind to both IL-4 and IL-13 and affects many processes including the immune response (15). (IL4R) signaling plays a pivotal role in various disease (16,17). The influence or relevance of the numerous mutant variations of the IL-4R gene in osteoporosis and other diseases is yet unknown, despite the fact that we have a reasonable understanding of the IL-4R's molecular processes. The rs1805010 variants are considered 'gain-of-function' mutations, where they abnormally enhance or promote STAT6 signaling (18). The obtained results of the serum IL-4R level indicate that there is a significant difference in women with osteoporosis compared to healthy women, and these results are consistent with previous study conducted (10). There is proof that polymorphisms in the functional candidate gene IL4R are linked to hand, knee, and hip osteoarthritis (19). As for the polymorphism of IL-4R, its effect on osteoporosis was not observed. This finding supports previous reports in rheumatoid arthritis (RA) patients that IL-4R SNP (rs1805010) may not be relevant as a risk factor for RA (20). While other studies found a link between two SNPs in the IL-4R a chain gene (rs1805013 and rs1805015) and hand osteoarthritis in the whole group of patients (21), and Gene interactions were discovered between the IL4R and TNF-alpha genes in patients with hand osteoarthritis (22). On the other hand, IL-4Rα polymorphisms alone may not always influence the susceptibility to disease (23). Combining IL-4Rα (rs1805010) SNP with mutant alleles in other genes may increase disease susceptibility. Multiple SNPs in several genes could act in concert or synergy to produce a significant effect (24).

The study had some limitations but the primary one was the small sample size of studied participants.

Conclusion: the current study indicated that there is a strong significant association between serum IL-4R level and the pathogenesis of osteoporosis. On the other hand, results noticed a non-significant association between IL-4R gene polymorphisms and osteoporosis.

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