

Prevalence of AGER Gene Polymorphism in Post Menopause Iraqi Sample with Osteoporosis and Osteopenia in type 2 Diabetes Mellitus

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Abstract

Osteoporosis (OP) is one of the most important metabolic disorders which is affected by interaction of genetic and environmental factors by almost 70% and 30% respectively. Genetic components are identified to strongly effect bone mineral density, bone building and turnover, so they play central role in determining risk of OP and fragility fractures. This case-control study consists of patient and control groups; Group A: (70) postmenopausal women with OP and osteopenia, Group B: (20) control group. five milliliters of blood sample were divided into three tubes; one tube (1ml) contain gel for obtain serum to measure glucose level, the others tubes containing ethylene-diamine-tetra-acetic acid (EDTA), in second tube 2ml stored in deep freeze at (-40 C°) until genomic analysis of DNA for the performance of PCR genotyping of gene polymorphisms of RAGE, in third tube 2ml used to perform Glycated Hemoglobin % (HbA1c) assays. HbA1c and Serum glucose levels is significantly increased in Group A. frequency of C allele and CC genotype of rs1800625 were high significant in group A (p<0.001) than control. A allele and AA genotype of rs1800624 were high significant in group A (p<0.001) than control. Homozygous 1800625 were (31%) and heterozygous 1800625(31%) compared to control homozygous 1800625 (5%) and heterozygous 1800625 (5%) respectively. Homozygous 1800624 were (28.5%) and heterozygous 1800624(28.5%) in group A compared to control homozygous 1800624 (5%) and heterozygous 1800624 (20%) respectively. In conclusion, the CC genotype and C allele of rs1800625 SNP and AA genotype and A allele of rs1800624 SNP can be considered as indicators for OP in post menopause Iraqi women with type2 DM.

Keywords: Osteoporosis, Diabetes mellitus, RAGE gene polymorphisms

تقييم نسبة انتشار تعدد اشكال جين AGER في العينة العراقية بعد انقطاع الطمث مع هشاشة وضعف العظام في مرضى السكري النوع الثاني

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الخلاصة

هشاشة العظام من أهم الاضطرابات الأيضية التي تتأثر بتفاعل العوامل الوراثية والبيئية بنسبة تقارب 70% و 30% على التوالي. تم تحديد المكونات الجينية للتأثير بقوة على كثافة المعادن في العظام، وبناء العظام لذلك فهي تلعب دوراً مهماً في تحديد مخاطر الإصابة بهشاشة العظام والكسور. تتكون هذه الدراسة من مجموعة المريض والمراقبة. المجموعة أ: (70) امرأة بعد سن اليأس مصابات بهشاشة العظام وضعف العظام، المجموعة ب: (20) مجموعة تحكم. تم تقسيم خمسة مليلتر من عينة الدم إلى ثلاثة أنابيب؛ أنبوب واحد 1 مل يحتوي على هلام للحصول على مصل لقياس مستوى الجلوكوز، والأنابيب الأخرى التي تحتوي على حمض الإيثيلين - ديامين - تترأ - أسيتيك، في الأنبوب الثاني 2 مل المخزن في تجميد عميق عند (-40 C°) لأجل التحليل الجيني للحمض النووي لأداء PCR لتعدد الأشكال الجينية لـ RAGE، في الأنبوب الثالث 2 مل لإجراء فحوصات نسبة الهيموجلوبين السكري (HbA1c). اظهرت النتائج ارتفاع مستويات الجلوكوز ونسبه الهيموجلوبين السكري في الدم بشكل كبير في المجموعة (أ) مقارنة بمجموعه التحكم. تم اكتشاف ارتفاع معدل انتشار RAGE تعدد الأشكال rs1800625 و rs1800624 في النساء بعد سن اليأس المصابات بهشاشة العظام في مرضى السكري النوع الثاني. كانت 1800625 متجانسة الزيجوت (31%) ومتغايرة الزيجوت (31%) مقارنة بالسيطرة 1800625 (5%) ومتماثلة الزيجوت (20%) مقارنة بالسيطرة 1800624 (28.5%) ومتغايرة الزيجوت (28.5%) في المجموعة أ مقارنة بالسيطرة 1800624 (5%) ومتغايرة الزيجوت (20%) مقارنة بالسيطرة 1800624 (20%) على التوالي. في الختام، قد يكون تعدد الأشكال rs1800625 و rs1800624 سبباً لخطر الإصابة بهشاشة العظام في النساء العراقيات بعد انقطاع الطمث المصابات بالسكري النوع الثاني

الكلمات المفتاحية: هشاشة العظام، مرض السكري النوع الثاني، تعدد الأشكال الجيني RAGE

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Introduction

Osteoporosis (OP) is one of the most important metabolic disorders which is affected by interaction of genetic and environmental factors by almost 70% and 30% respectively ⁽¹⁾. Genetic components are identified to strongly effect bone mineral density (BMD), bone building and turnover, so they play a central role in determining risk of OP and fragility fractures ⁽²⁾. Diabetes mellitus (DM) is a common health problem worldwide counting about 1.2 million cases in Iraq in 2015, Type 2 DM (T2DM) represents about 90 to 95% of the overall diabetes types worldwide ⁽³⁾. Leidig-Bruckner *et al.* (2014) described prevalence of osteoporosis in women with T2DM was 21.9% and overall prevalence of low trauma fractures 5.7% ⁽⁴⁾. Hyperglycemia can reduce the bone density through different pathways. Toxic effects initiated by high serum glucose levels can directly reduction the function and number of osteoblasts ⁽⁵⁾. In fact, advanced glycation end products (AGEs) are dangerous heterogeneous compound of irreversible products resulting from non-enzymatic glycation. This Reactions take place between the reactive carbonyl group of a reducing sugar, nucleic acids, lipids or proteins. The source of AGEs can be formed endogenously or exogenously under normal and pathological conditions ⁽⁶⁾. AGEs show a main role in the development of OP, osteopenia and are associated with aging and hyperglycemia ⁽⁷⁾. A receptor advanced glycation end products also known as RAGE is type I cell surface receptor for AGEs belongs to the immunoglobulin "Ig" superfamily and has been called as a pattern recognition receptor PRR ⁽⁸⁾. The RAGER gene is located on the small arm of chromosome 6 "6p21.3" and the genomic sequence of this gene is polymorphic. This locus is associated in inflammatory and immune responses and is also the locus of main histocompatibility complex III (MHC. III). Single nucleotide polymorphism "SNP" is defined as a best common kind of polymorphism in which the change occurs in one nucleotide in sequence of DNA, some previous studies show association between SNPs variation and OP ⁽⁹⁾. Therefore, the objective of this study was to evaluate the prevalence phenotype of RAGE and evaluate its relationship in a sample of Iraqi post menopause with osteoporosis and osteopenia in type2DM.

Material and Methods

Study design (case-control study) made over 4 months' period from march till June 2021 at Medical city –Baghdad Teaching Hospital - Rheumatology and Rehabilitation Consultation Unit. This study consists of patient groups, this group were composed of (70) Postmenopausal Women with osteoporosis and osteopenia. In addition, to control group (20) as Healthy women without osteoporosis or osteopenia. The age of subject is ≥ 45 years. Disease female will be

diagnosing as Osteoporosis and Osteopenia according to World Health Organization (WHO). The selection and diagnosis of patient will be doing under the supervision of rheumatologist physician in Baghdad Medical City. The current study protocol was approved by the local ethical committee of the College of Pharmacy, Baghdad University. Exclusion criteria:

- Cushing's syndrome, hyperparathyroidism, thyrotoxicosis.
- Gastrointestinal tract diseases: Including ulcerative colitis, celiac disease and inflammatory bowel disease.
- Renal disease and epileptic
- Multiple myeloma, mastocytosis, lymphoma and leukemia. Inherited disorders: including osteogenesis imperfecta, Marfan's syndrome, hemochromatosis.
- Rheumatic disorder such as Rheumatoid arthritis and Ankylosing Spondylitis.
- Medication that can influence bone metabolism as steroid, Vitamin D and hormone replacement therapy.

After clinical examination, five ml of blood sample were collected from each subject in population study and divided into two parts: the first 3 ml of blood put in EDTA tubes for genotyping, and for perform Glycated Hemoglobin % (HbA1c) assays, while the second part 2 ml was used for serum assessment of glucose tests. Serum glucose level can be evaluated using a ready-made kit for this purpose, according to the method of Barham and Trindor (1972) ⁽¹⁰⁾. Glycated hemoglobin level was evaluated using a ready-made kit for this purpose according to Abraham *et al* (1978) ⁽¹¹⁾.

Determination of AGER Gene Polymorphism

Genomic DNA was isolated from Blood sample according to the protocol of ABIO pure Extraction. Then genotyping depends on the analysis of promoter region of RAGE by utilizing PCR amplification and then sequencing according to method described by Sanger and Coulson 1975 ⁽¹²⁾. The primers used for amplify fragment of 797 bp of AGER gene are:

AGER-F 5'-
TGTA AACGACGGCCAGTGAAGAATGGGA
AGGGAGTTATT-3`
AGER-R 5'-
CAGGAAACAGCTATGACAAGAGTCCTTCAG
GTACTAGAG-3`

The PCR amplification was carried out with 3 ng/ μ l of genomic DNA, 2 μ l of master mix, 10 μ l of each primer, 7.5 μ l nuclease free water. PCR cycles had been carried out as illustrated in (table 1). The PCR product then applied for gel electrophoresis and after that was sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received and analyzed using genius software.

Table 1. PCR amplification program

Steps	Temperature °C	Time (m: s) M=minute, S=second	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	
Hold	10	10:00	1

Statistical Analysis

The results were analyzed by Statistical Package for the Social Sciences (SPSS) (version 20.0) program and Minitab (version18). One-way ANOVA and t-test were used for the comparison of mean \pm standard deviation (SD) of biochemical parameters of the patient and control groups and among the genotypes of RAGE polymorphism. Alleles and genotypes frequency besides of odds ratios (OR) and their 95% confidence intervals (CI) of the patients and control group were determined to utilize Pearson's chi-square test. The results of the analysis with a P value < 0.05 were considered significant and $p < 0.01$ as highly significant.

Sequencing Results Insertion**Serum glucose and HbA1c levels**

Table 1 presents the mean \pm SD of serum glucose and HbA1c level obtained from 70 diabetic post menopause Iraqi women with osteoporosis and osteopenia beside to 20 women as a control group. In Group A, the serum glucose levels were significantly higher (146.13 ± 12.79) compared to healthy control (84.56 ± 10.25). Also, HbA1c levels were significantly higher compared (6.89 ± 0.29) to healthy control (5.15 ± 0.737).

Table 2. Comparison between characteristic and biochemical parameters of the study groups

Data	Group A N= (70)	Group B N=(20)	P value
Age	57.57 ± 6.68	57.15 ± 5.47	0.775
Serum glucose(mg\dl)	146.13 ± 12.79	84.56 ± 10.25	< 0.0001
HbA1C(%)	6.89 ± 0.29	5.15 ± 0.737	< 0.001
Ethnicity			
Arab	68(97%)	19(95%)	
Kurd/Turkman	2(3%)	1(5%)	

P value < 0.05 considered significant and P value < 0.01 considered high significant

Genotypes and alleles frequency of RAGE

After analysing PCR products of the AGER polymorphism, 4 types of SNP detected rs1800625 (-429T/C), rs1800624 (-374T/A), rs118122061 and rs143118560 as seen in Figures (1,2&3). Tables (3) lists the genotype and allele frequencies in % and the number of patients (No) having each genotype of the study groups. The distribution of genotypes in women with Group A and control group was an agreement with Hardy Weinberg equilibrium. We detected a significant difference (P value < 0.001) between the frequency of genotypes and alleles of rs1800625 (-429T/C), rs1800624 (-374T/A), in the patients Group A compared with control group. The gene and allele frequencies for rs1800625 variant were TT 38%, TC 31%, CC 31%, (T53%, C47%) for group A, TT90%, TC 5%, CC 5% (T 92.5%, C 7.5%) for control healthy group. while The gene and allele frequencies

for rs1800624 variant were TT 43%, TA28.5%, AA28.5%(T57%, A43%) for group A, TT75%, TA20%, AA5% (T85%, A15%) for control group. In other hand, there was non-significant difference (p-value < 0.05) between the frequency of genotypes of AGER gene variants rs118122061 and rs143118560 in the women with group A as compared to control group while allele of AGER gene variant rs143118560 were significant (p-value > 0.05) and allele of AGER gene variant rs118122061 non-significant. The gene and allele frequencies for rs11812261 variant were GG 79%, GA 21%, (G89%, A11%) for group A, GG85%, GA 15% (G 92.5%, A 7.5%) for control group, while The gene and allele frequencies for rs143118560 variant was AA51%, AT26%, TT23%(A64%, T36%)for group A, AA75%, AT15%, TT10% (A82.5%, T17.5%) for control group.

Table 3. Comparison between genotypes and alleles frequency of RAGE polymorphism of the study entire group A

SNP	Group A N=(70)			Healthy control N= (20)				
	Genotypes	NO.	%	NO.	%	P Value	OR	95% IC
rs1800625 (-429T/C)	TT	26	38%	18	90%	<0.001	15.23	4.18-55.4
	TC	22	31%	1	5%		15.23	4.18-55.4
	CC	22	31%	1	5%			
Allele frequency		NO.	%	NO.	%	P Value		
T		74	53%	37	92.5%	<0.001	11	3.02-40.1
C		66	47%	3	7.5%			
rs1800624 (-374T/A)	TT	30	43%	15	75%	0.025	2.5	0.68-9.11
	TA	20	28.5%	4	20%		10	2.7-36.42
	AA	20	28.5%	1	5%			
Allele frequency		NO.	%	NO.	%	P value		
T		80	57%	34	85%	0.001	4.25	1.16-15.48
A		60	43%	6	15%			
rs118122061	GG	55	79%	17	85%	0.526	1.54	0.422-5.6
	GA	15	21%	3	15%			
	AA	0	0	0	0			
Allele frequency		NO	%	NO	%	P value		
G		125	89%	37	92.5%	0.146	1.48	0.41-5.39
A		15	11%	3	7.5%			
rs143118560	AA	36	51%	15	75%	0.167	2.5	0.68-9.1
	AT	18	26%	3	15%		3.33	0.9-12.13
	TT	16	23%	2	10%			
Allele frequency		NO	%	NO	%	P Value		
A		90	64%	33	82.5%	0.03	2.61	0.71-9.5
T		50	36%	7	17.5%			

p>0.05 is non-significant<0.05 significant and p<0.01 high significant.

Relationship between AGER Polymorphism and Serum Glucose and HbA1c Levels

The results are given in Table 3 shows the relationship between AGER polymorphism and biochemical levels (Glucose and HbA1c) in Group A. For rs1800625 SNP, Glucose level of CC genotype (147.27±14.94) were higher than TT (144.5±10.93) and TC (146.91±12.94) genotype respectively, but non-significant, also TC (146.91±12.94) were higher than TT (144.5±10.93) but still non-significant. While HbA1c level of TT genotype (6.93±0.348) were higher than CC (6.91±0.222) and TC (6.84±0.29) respectively, but non-significant. For rs1800624SNP, Glucose level of TT genotype (150.83±12.26) were higher than AA (143.8±13.35) and TA (141.4±11.07);

respectively but statistically non-significant, HbA1c level of TT genotype (7.00±0.312) were higher than AA (6.88±0.338) and TA (6.93±0.302) respectively, but non-significant. For rs118122061SNP, glucose level of GG (147.24±13.06) were higher than GA (142.07±11.27), but non-significant and HbA1c level of GG genotype (6.9±0.26) were higher than GA (6.85±0.37), but also non-significant. For rs143118560SNP, our data reported glucose level of AA (145.56±11.67) were higher than TT (143.5±13.55) and lower than AT (147.56±12.61) respectively but also still non-significant and HbA1c level of AA genotype (6.8±0.24) were lower than TT (6.92±0.33) (non-significant) and AT (7.00±0.32) but significant.

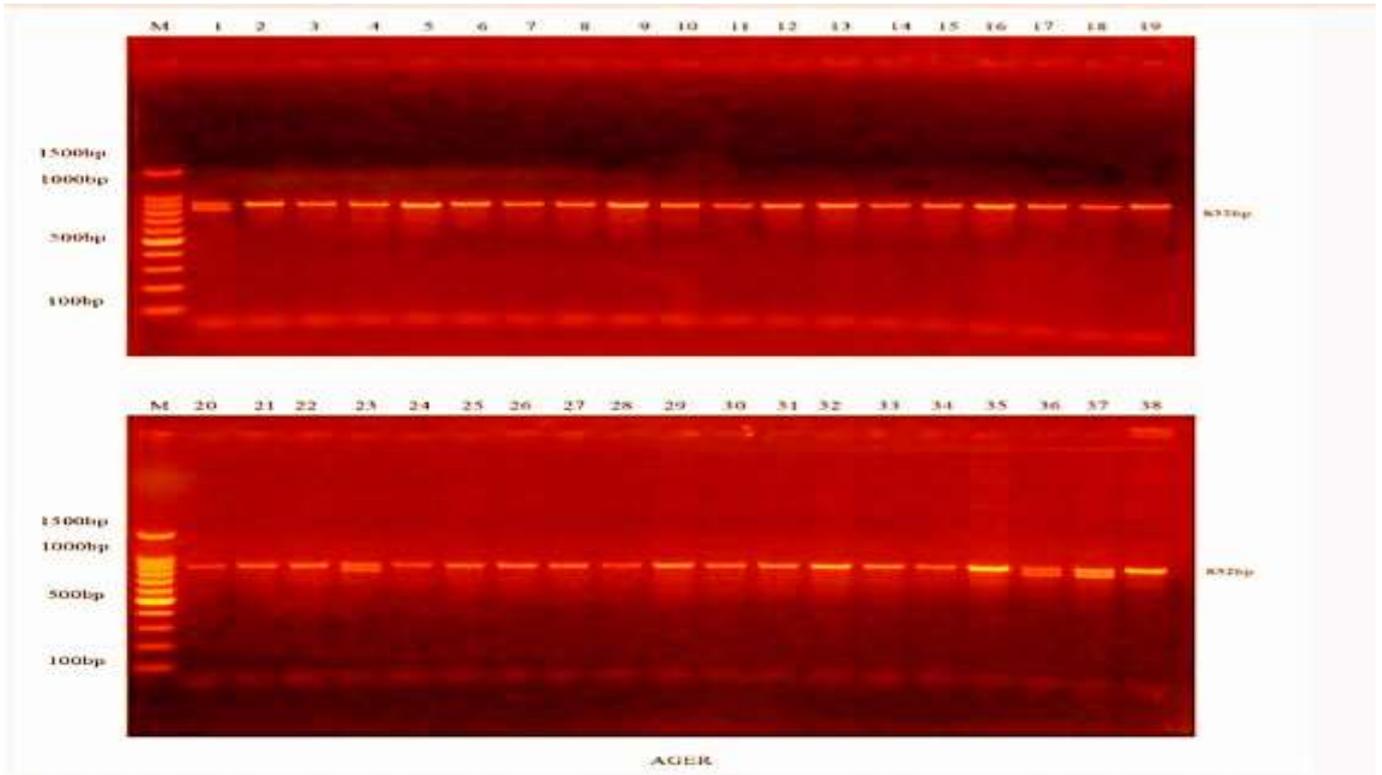


Figure 1. Gel electrophoresis for PCR product of AGER: The results of the amplification of AGER specific gene region of Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-38 resemble 832bp PCR products.

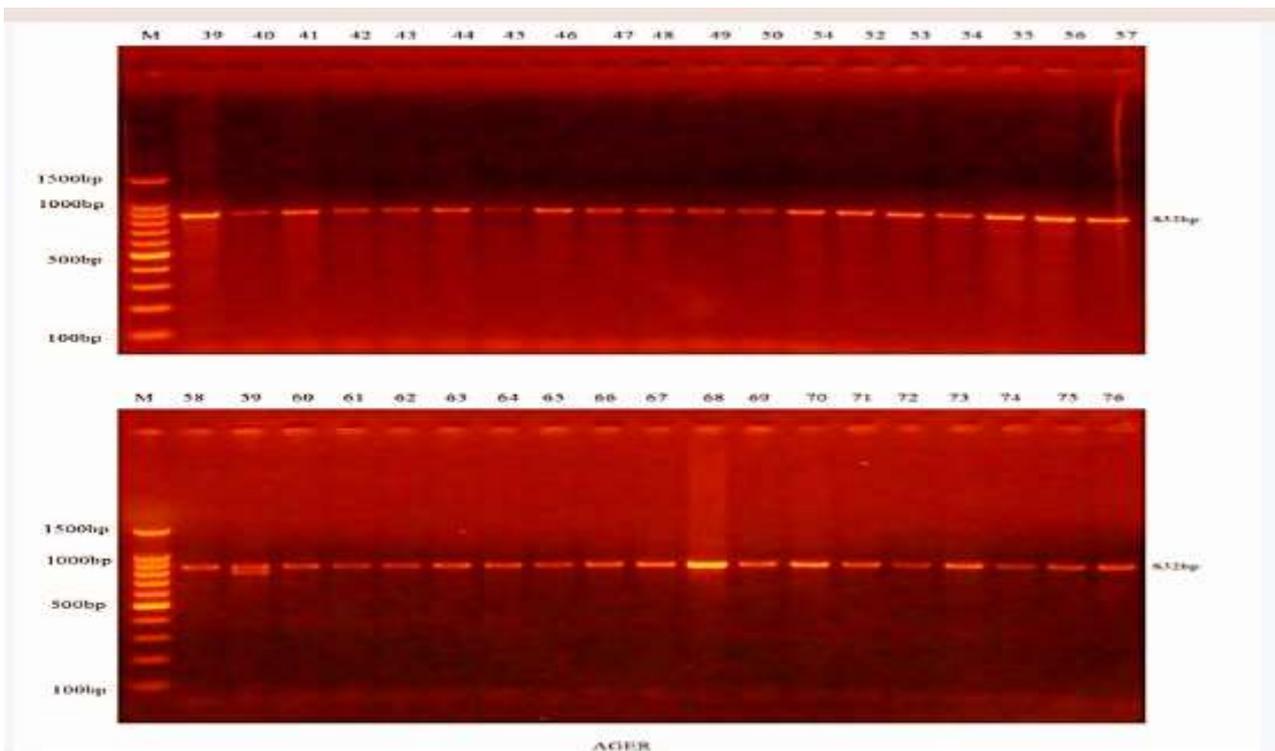


Figure 2. Gel electrophoresis for PCR product of AGER: Results of the amplification of AGER specific gene region of Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 39-76 resemble 832bp PCR products

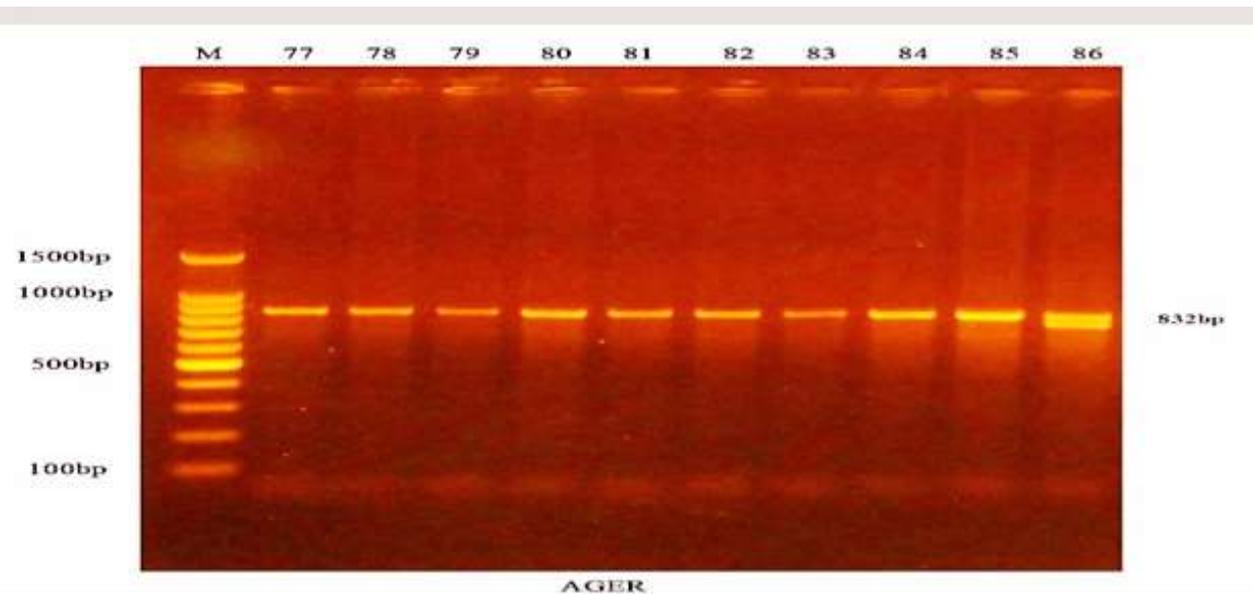


Figure 3. Gel electrophoresis for PCR product of AGER: Results of the amplification of AGER specific gene region of Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 77-86 resemble 832bp PCR products

Table 4. Comparison between biochemical parameters of the patients group A according to AGER genotypes

SNP. rs1800625	Parameter	TT No.26	CC No.22	P value	TT No.26	TC No.22	P value
	Glucose	144.5±10.93	147.27±14.94	0.462	144.5±10.93	146.91±12.94	0.488
	HbA1c	6.93±0.348	6.91±0.222	0.803	6.93±0.348	6.84±0.29	0.342
		TC No.22	CC No.22	P Value			
	Glucose	146.91±12.94	147.27±14.94	0.932			
	HbA1c	6.84±0.29	6.91±0.222	0.387			
SNP. rs1800624	Parameter	TT No.16	AA No.12	P value	TT No.16	TA No.12	P value
	Glucose	150.83±12.26	143.8±13.35	0.061	150.83±12.26	141.4±11.07	0.008
	HbA1c	7.00±0.312	6.88±0.338	0.353	7.00±0.312	6.93±0.302	0.576
		TA No.12	AA No.12	P value			
	Glucose	141.4±11.07	143.8±13.35	0.54			
	HbA1c	6.93±0.302	6.88±0.338	0.706			
SNP. rs118122061	Parameter	GG No.22	GA No.15	P value			
	Glucose	147.24±13.06	142.07±11.27	0.167			
	HbA1c	6.9±0.26	6.85±0.37	0.532			
SNP. rs143118560	Parameter	AA No.36	TT No.16	P value	AA No.36	AT No.18	P value
	Glucose	145.56±11.67	143.5±13.55	0.579	145.56±11.67	147.56±12.61	0.566
	HbA1c	6.8±0.24	6.92±0.33	0.216	6.8±0.24	7.00±0.32	0.024
		AT No.18	TT No.16	P value			
	Glucose	147.56±12.61	143.5±13.55	0.373			
	HbA1c	7.00±0.32	6.92±0.33	0.479			

Discussion

Although several investigators have long addressed the question of how DM induces osteopenia and osteoporosis, the exact underlying mechanism is still unknown. Many factors include food availability, absence of exercise, low sun exposure, gender, age, several diseases, inflammation, genetic polymorphism and ethnic group associated with development of osteoporosis. Therefore, various investigated tried to know the etiology of osteoporosis or as a minimum identify its risk factor⁽¹³⁾. The BMD levels in type 2DM patients were normal or high, several investigators reported a negative effect of type 2 DM on BMD. For instance, Yaturu and colleagues found a significantly low BMD of hip in type 2 DM patients when compared to age-matched normal individuals⁽¹⁴⁾. The current study shows a significant difference in serum levels of glucose when a comparison among Group A (146.13 ± 12.79) and control group (84.56 ± 10.25), also significant difference found in levels of HbA1c (%) in Group A (6.89 ± 0.29) when compared to control group (5.15 ± 0.737), as shown in table 2 all this result was agreement with recent study⁽¹⁵⁾ ⁽¹⁶⁾. It is commonly believed that hyperglycemia is a salient factor that has direct and indirect deleterious effects on osteoblast function, bone formation and also on proliferation and differentiation of mesenchymal stem cells⁽¹⁷⁾. RAGE have a role in regulating bone homeostasis under physiological disorders and may implicated in several bone-associated diseases such as OP and osteopenia however, the particular cell type that facilitates signaling of RAGE, and the downstream effects of RAGE activation on bone homeostasis and pathology, still unclear⁽¹⁸⁾. This study is considered the first study that estimate the prevalence of the RAGE gene polymorphism in Iraq. There are no studies in the neighboring countries that could be compared to the results and analyzing the impact of genes polymorphisms on the development of OP in Type 2 DM have been carried out; however, we compared the findings of this study to studies conducted on American, European, Asia and African populations. Several lines of evidence reported a role for RAGE and its ligands in stimulating osteoclastic activity and osteoclast maturation. In addition, HMGB1 (high mobility group box 1)-RAGE signaling is concerned in recruitment of osteoclasts, osteoblasts, and blood vessels through bone formation. AGE-RAGE signaling may show an adverse role in osteoblast differentiation and function⁽¹⁹⁾. Several other studies have recommended that RAGE levels may actually be raised in OP, osteopenia and DM with high levels of bone and cartilage turnover⁽²⁰⁾ ⁽²¹⁾. In the current study we reported the occurrence of polymorphisms in the promoter region of RAGE and identified a number of key polymorphisms. The results of our study showed that RAGE gene

rs1800625 and rs1800624 polymorphisms were statistically different between Group A compared to control group. RAGE gene rs118122061 and rs143118560 polymorphisms were non-significant between Group A compared to control, as exposed in Table 3 and Figures (1,2 and3). These data suggest that the polymorphisms involved in alterations in RAGE gene regulation that can influence on pathogenesis of DM post menopause women with OP and osteopenia. Prior studies that have noted the important role of race and ethnic on the epidemiology of osteoporosis⁽²²⁾. Therefore, our results agree with Ying several studies in the chinese population⁽²³⁾ ⁽²⁴⁾ and disagree with Raska *et al* study that found diabetes-specific parameters in addition to RAGE polymorphisms did not associate with BMD or fractures in T2DM postmenopausal women⁽²⁵⁾. The SNPs rs1800625 and rs1800624 was found to rise the transcription activity of AGER in vitro. They involve an increase in RAGE expression, which might influence the pathogenesis of several inflammatory diseases and DM⁽⁹⁾. Result in table 4 shown biochemical parameters of the patient's group according to RAGE genotypes, the women with rs 1800625 CC genotype had high mean \pm SD of glucose levels (147.27 ± 14.94) compared to TT (144.5 ± 10.93) and TC (146.91 ± 12.94) but still non-significant. Also, the women with rs1800624 TT (150.83 ± 12.26) genotype had high mean \pm SD of glucose levels compared to AA (143.8 ± 13.35) and TA (141.4 ± 11.07), while women with rs118122061 GG (147.24 ± 13.06) genotype had high mean \pm SD of glucose levels than GA (142.07 ± 11.27) genotype. In other hand women with rs143118560 AT genotype had high mean \pm SD (147.56 ± 12.61) compared to TT (143.5 ± 13.55) and AA (145.56 ± 11.67) genotype. the women with rs 1800625 TT genotype had high mean \pm SD of HbA1c levels (6.93 ± 0.348) compared to CC (6.91 ± 0.222) and TC (6.84 ± 0.29). Also the women with rs1800624 TT (7.00 ± 0.312) genotype had high mean \pm SD of HbA1c levels compared to AA (6.88 ± 0.338) and TA (6.93 ± 0.302), while women with rs118122061 GG (6.9 ± 0.26) genotype had high mean \pm SD of HbA1c levels compared to GA (6.85 ± 0.37). in other hand women with rs143118560 AA (6.8 ± 0.24) had lower mean \pm SD compared to TT (6.92 ± 0.33) (non-significant) and AT (7.0 ± 0.32) (significant), no statistical significance was found between glucose and HbA1 levels with osteoporosis except in SNP rs143118560 AA and AT genotype of HbA1c levels, and SNP 1800624 TT and TA genotype of glucose levels so absent clear effect of RAGE polymorphism on biochemical levels of glucose in women with osteoporosis, because glucose homeostasis affected by a variety of factors, Glucose homeostasis is maintained by a complex neuro hormonal system, which modulates glucose uptake, glucose assembly, and exogenous glucose utilization following food ingestion⁽²⁶⁾ ⁽²⁷⁾. There are several hormones

participate in glucose metabolism include insulin, glucagon, amylin, glucagon-like peptide-1 (GLP-1), epinephrine, cortisol, and growth hormone (GH). These hormones control glucose levels and act on several target tissues, involving muscle, liver, adipocyte⁽²⁸⁾.

Conclusion

In the current study, a high prevalence of RAGE polymorphism rs1800625 and rs1800624 were detected in postmenopausal women with osteoporosis in type 2DM. Homozygous 1800625 were (31%) and heterozygous 1800625 (31%) compared to control homozygous 1800625 were (5%) and heterozygous 1800625 were (5%) respectively. Homozygous 1800624 were (28.5%) and heterozygous 1800624 (28.5%) compared to control homozygous 1800624 were (5%) and heterozygous 1800624 were (20%) respectively. Thus, the rs1800625 and rs1800624 polymorphism might be a causal risk allele for osteoporosis in postmenopausal Iraqi women with type 2 DM, also so it might be used as a biomarker. Further research should be undertaken to confirm these results by suggesting more studies should be using a larger number of samples in different cities of the Iraq.

References

1. Tamer A, Gheita H, and Nevin H. Epidemiology and awareness of osteoporosis: a viewpoint from the Middle East and North Africa. *Int. J. Clin. Rheumatol.* 2018; 13(3): 134-147.
2. Rivadeneira F, Mäkitie O. Osteoporosis and bone mass disorders: from gene pathways to treatments. *Trends Endocrinol Metab.* 2016; 27:262–281.
3. Hussein EA, Kadhim DJ and Al-Auqi TF. Belief about medications among type 2 diabetic patients attending the national diabetes center in Iraq. *Iraqi J Pharm Sci*, 2017;26(2).
4. Leidig-Bruckner G, Grobholz S, Bruckner T, et al. Prevalence and determinants of osteoporosis in patients with type 1 and type 2 diabetes mellitus. *BMC Endocr Disord*, 2014; 14:33.
5. Ali IA, and Ali SH. Impact of osteocalcin level on vascular calcification in Type 2 diabetics in relation to fibroblast growth factor-23(FGF-23) *Iraqi J Pharm Sci*, 2018;27(2).
6. Rungratanawanich W, Qu Y, Wang X et al. Advanced glycation end products (AGEs) and other adducts in aging-related diseases and alcohol-mediated tissue injury, *Experimental & Molecular Medicine* 2021;53:168–188.
7. Yang D, Chiang T, Chang I, et al. Increased levels of circulating advanced glycation end-products in menopausal women with osteoporosis, *International Journal of Medical Sciences* 2014; 11(5): 453-460.
8. Otta C, Jacobsb K, Hauckec E, et al. Role of advanced glycation end products in cellular signaling, *Redox Biology*, 2014; 411–429.
9. Serveaux-Dancer M, Jabaudon M, Creveaux I, Belville C, et al. Pathological implications of receptor for Advanced Glycation End-Product (AGER) gene polymorphism, *Hindawi Disease Markers Volume* 2019; 17: 2067353.
10. Barham D, Trinder P. An improved color reagent from the determination of blood glucose by the oxidative system. *Analyst.* 1972; 97: 142-145.
11. Abraham EC, Huff TA, Cope ND, et al. Determination of the glycosylated hemoglobin (HbA1) with a new micro column procedure, suitability of the technique for assessing the clinical management of diabetes mellitus. *Diabetes* 1978; 27(9): 931-937.
12. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Mol Biol* 1975; 94:44.
13. AL-Azzawie AF, Husain WN, Salih MH, AL-Assie AH. The relationship between some electrolyte levels and MTHFR C667T gene polymorphism, *Eurasia J Biosci* 2020;14, 545-551
14. Yaturu S, Humphrey S, Landry C, Jain SK. Decreased bone mineral density in men with metabolic syndrome alone and with type 2 diabetes. *Med Sci Monit* 2009; 15: CR5-CR9.
15. Deng X, Xu M, Shen M, Cheng J. Effects of type 2 diabetic serum on proliferation and osteogenic differentiation of mesenchymal stem cells. *J Diabetes Res.* 2018:1-9.
16. Zhou Y, Li Y, Zhang D, Wang J, and Yang H, "Prevalence and predictors of osteopenia and osteoporosis in postmenopausal Chinese women with type 2 diabetes," *Diabetes Research and Clinical Practice*, 2010;(90);3:261–269.
17. Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms. *World J Diabetes* 2011; 2(3): 41-48.
18. Zhou Z, Xiong WC: RAGE and its ligands in bone metabolism. *Front Biosci (Schol Ed)*. 2011 Jan 1; 3:768-76.
19. Zheng Z, Wen-Cheng X. RAGE and its ligands in bone metabolism, *Frontiers in Bioscience* ,2011; S3:768-776.
20. Galliera E, Marazzi MG, Gazzaruso C, Gallotti P, et al. Evaluation of circulating sRAGE in osteoporosis according to BMI, adipokines and fracture risk: a pilot observational study. *Immun Ageing* 2017; 14:13.
21. Dachun Y, and Michael B, Hyperglycemia-induced reactive oxygen species increase expression of the receptor for Advanced

- Glycation End Products (RAGE) and RAGE Ligands *Diabetes* 2010; 59(1): 249-255.
22. Al Anouti F, Taha Z, Shamim S, Khalaf K, et al. An insight into the paradigms of osteoporosis: From genetics to biomechanics. *Bone reports*, 2019;100216.
 23. Ying Z, Nan J, Feng H, Naijun Fan, X et al. Association of single-nucleotide polymorphisms in the RAGE gene and its gene-environment interactions with diabetic nephropathy in Chinese patients with type 2 diabetes *Oncotarget*, 2017;8:(57): 96885-96892.
 24. Kang P, Tian C, Jia C. Association of RAGE gene polymorphisms with type 2 diabetes mellitus, diabetic retinopathy and diabetic nephropathy. *Gene*. 2012; 500:1–9.
 25. Raska I Jr, Raskova M, Zikan V, Skrha J. Prevalence and risk factors of osteoporosis in postmenopausal women with type 2 diabetes mellitus. *Cent Eur J Public Health* 2017; 25:3-10.
 26. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and glucagon, *Diabetes Spectr*, 2004;17:183–90.
 27. Szablewski L. *Glucose Homeostasis and Insulin Resistance*, Bentham Science Publishers; 2011.
 28. Jim Parker. glucose metabolism, energy production and regulation of cellular and whole-body metabolism, *Journal of the Australasian College of Nutritional and Environmental Medicine Inc (ACNEM)*2020;39(1).



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