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Molecular Study of COL1A1 Gene in Women with Osteoporosis in Mosul City

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Abstract:
Among the most prevalent diseases in the world is osteoporosis, and it is identified by the changes that occur in bone mineral density according to international health standards, and the disease is diagnosed by dual-energy X-ray DEXA. Osteoporosis is divided into two types (primary and secondary), where the primary type is in elderly men and women due to aging and in women in menopause. As for secondary osteoporosis, it is the result of different diseases and treatments, or because of tumors, cancerous diseases, systemic diseases, and endocrine diseases. Different diets, wrong diets, and lack of exercise are all causes of osteoporosis.
Between September and November of the year 2021, private pathological analysis laboratories in Mosul reviewed (96) women with ages ranging from (45 to 35) years. Based on the disease's clinical cases, samples were chosen. The samples were separated into two groups in accordance with the outcomes of the biochemical analysis: The first category: 25 women who had no problems made up this group, which acted as a control group. The second group: Biochemical results revealed that 71 women in this group had osteoporosis. The results for women with osteoporosis for the COLA1 gene showed that the frequency value of the mutated genotype AA in the group of women with osteoporosis was 10%, which is the lowest compared to the mutated genotype in the control group of 5%, while the value of the healthy (normal) AA genotype was the same. The highest rate was Compared to the healthy group, where the percentage of individuals with a healthy (normal) genotype was more than 90%, there were 62% more women with osteoporosis.
Regarding the heterogeneous genotype AC, there were 28% more observations in the group of infected women than there were in the 5% control group. In terms of allelic recurrence, the findings revealed that the patients' group had a higher incidence of the mutant allele A 23% compared to 8% in the control group. Natural allele prevalence in patients was 62%, as opposed to 92% in the control group. It turns out that we have a wide variety of genetic variants, which can be further broken down into two main categories: transition variations and (Transversion) variations. The locations of these variations depend on the type of heterogeneous bases. The study's findings also revealed a genetic link between variances and several genotypes and biochemical indicators of osteoporosis.
Keywords: T-ARMS-PCR, COLA1 Gene, Polymorphism, Osteoporosis

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دراسة جزيئية لجين الكولاجين 1 للنساء المصابات بهشاشة العظام في مدينة الموصل

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الملخص
يعد مرض هشاشة العظام من أكثر الامراض انتشارا في العالم، ويتم تحديده من خلال التغيرات التي تحدث في كثافة المعادن في العظام وفقا للمعايير الصحية العالمية، ويتم تشخيص المرض بالأشعة السينية ثنائية الطاقة DEXA. وتقسم هشاشة العظام الى نوعين (أولي وثانوي)، حيث يكون النوع الأولي الأساسي عند كبار السن من الرجال والنساء بسبب الشيخوخة وعند النساء في سن اليأس، أما هشاشة العظام الثانوية فهي ناتجة عن أمراض وعقاقير مختلفة أو بسبب الأورام او الأمراض السرطانية والأمراض الجهازية، والأنظمة الغذائية الخاطئة وعدم ممارسة الرياضة جميعها تؤدي الى تكوين هشاشة العظام. بين شهري أيلول وتشرين الثاني من عام 2021 تم مراجعة المختبرات الاهلية في الموصل من قبل النساء المصابات بهشاشة العظام اللواتي تتراوح اعمارهن بين ال 35 الى 45 سنة، حيث تم أخذ العينات بناء على الحالات السريرية للمرض. تم فصل العينات الى مجموعتين وفقا لنتائج التحليل: الفئة الأولى 25 امرأة لم يكن لديهن أي مشاكل مرضية حيث عدت مجموعة السيطرة بينما الفئة الثانية المكونة من 71 امرأة مصابة بهشاشة العظام. أظهرت النتائج للنساء المصابات بهشاشة العظام بالنسبة للجين COLA1 بان قيمه تكرار النمط الوراثي الطافر AA لدى مجموعه النساء المصابات بهشاشة العظام كانت بنسبه 10% وهي الأقل مقارنة بالنمط الوراثي الطافر لدى مجموعة السيطرة بنسبة 5% في حين أن قيمة النمط الوراثي السليم (الطبيعي) AA كانت هي الأعلى لدى النساء المصابات بهشاشة العظام بنسبة 62% مقارنة بمجموعة الاصحاء التي كان فيها نسبه النمط الوراثي السليم (الطبيعي) عالية 90% اما بالنسبة للنمط الوراثي المتباين AC في مجموعه النساء المصابات 28% كانت نسبة المشاهدات عالية مقارنة بمجموعها السيطرة 5 %. أما بالنسبة للتكرار الأليلي فقد بينت النتائج بان نسبه المشاهدة للأليل الطافر A كانت مرتفعة في مجموعه المرضى 23% مقارنه بمجموعه السيطرة 8% أما بالنسبة للأليل الطبيعي فقد كانت نسبته لدى المرضى 62%مقارنه مع مجموعه السيطرة 92%.

الكلمات المفتاحية: تفاعل سلسلة متعدد البلمرة، جين كولاجين 1، تعدد الاشكال، هشاشة العظام

Introduction

Worldwide, osteoporosis affects about 200 million people and is characterized by reduced bone density and increased fragility [2]. Osteoporosis consequently causes more than 8.9 million fractures per year, or one fracture every three seconds [3]. The fact that 20% of bone fractures in elderly people result in death within a year of the injury only serves to highlight how severe this disease is [4]. Within the first year after suffering an osteoporotic bone fracture, more than half of patients endure loss of function and need assisted living. [5]. In addition, osteoporosis medications in the US come with a significant financial burden and have adverse effects. The average yearly healthcare expense in the U.S. for osteoporosis and senior fractures was $16 billion in 2002 [6]. Gender is a significant risk factor for the development of osteoporosis, which affects women more severely because of the loss of estrogen following the onset of menopause. However, slow bone loss and an increased risk of osteoporosis are also present in men over the age of 50. Thus, 39% of all cases of osteoporosis-related fractures occur in men [7]. People who have osteoporosis experience both higher rates of bone resorption and impaired osteogenesis. The majority of osteoporosis treatments currently available concentrate on anti-resorptive techniques to stop additional bone loss. These include medications like denosumab and bisphosphonates, which may cause unintended adverse effects. [8].

Osteoporosis is split into two types (primary and secondary), with the primary kind affecting older people due to aging and menopause in women. When it comes to secondary osteoporosis, it is brought on by many conditions and therapies, as well as by tumors, malignant conditions, systemic illnesses, and endocrine disorders.[9]

Osteoporosis-related fractures considerably increase morbidity and mortality worldwide. Although secondary osteoporosis can afflict up to 30% of postmenopausal women, > 50% of premenopausal women, and 50% to 80% of men, postmenopausal osteoporosis is the most common kind.[10]

COLA1 is one of the genes affecting osteoporosis, as this gene encodes the protein α1(I) of the first type, which is the main protein of bone This protein affects the bone structure and consists of two parts of a chain and part of a chain to be tripartite Snail, the occurrence of genetic mutations in the COLA1 gene leads to a defect in the formation of chains and the decoupling of the spiral structure, which leads to weak collagen and thus the occurrence of osteoporosis[11] Mutations in this gene can also affect other tissues rich in collagen, leading to disorders such as dental defects and hearing impairment[12] dramatic consequences of changes to this gene's coding regions. Subtle variations in expression may be caused by changes in its regulatory regions. Differences in bone mineral density and fracture risk are caused by genetic responses to extracellular signals. As a result, it has been discovered that a G/T polymorphism in the gene's intron 1 inside the recognition region of the transcription factor Sp1 is connected to bone mineral density (BMD) at both the spinal and femoral neck sites. [13]

Pro-alpha-1 type 1 collagen, which is most prevalent in bone and is produced in a 1:2 ratio with pro-alpha-2 collagen, is encoded by the COL1A1 gene. The COL1A1 G-1997T (rs1107946) genetic variant is likely to have

an impact on transcription factor binding, which will change gene expression and protein synthesis. As a result, the 2:1 ratio change, bone strength declines, and porosity rises, all of which increase the risk of fractures. [14]

Materials and method

Case Study: The current study involved (96) women with ages ranging from (45-35) years who were reviewed by private pathological analysis laboratories in Mosul between September and November of the year 2021. Samples were chosen based on clinical cases of the disease. Based on the biochemical results, the samples were split into two groups:

The first group was made up of 25 healthy women who served as the control group because they had no health issues.

The second group contained 71 women who, according to biochemical findings, had osteoporosis

Collection of Blood sample

In order to extract DNA, 5 ml of venous blood from these patients was divided into two groups. The first group was put into tubes containing EDTA anticoagulant, and the second group was put into tubes without any anticoagulant. The blood was allowed to coagulate in the tubes for an hour before the blood was centrifuged for ten minutes at a speed of three thousand cycles per minute to get the blood serum for the biochemical testing.

DNA extraction:

Using a modified version of the technique described by (Iranpur and Esmailizadeh 2010), DNA was extracted from the blood of (71), control group patients who were included in this investigation.

Genotyping:

Tetra–ARMS-PCR Reactions:

After being measured by biodrop, the DNA concentration in each study sample was adjusted by dilution with TE buffer solution to reach the necessary concentration for running PCR reactions, which was (25) ng/microliter for each sample Four primers are inserted for each primer reaction (F-outer and R-outer) throughout the entire gene. For the mutant allele, forward outer-reverse inner is used instead of the normal allele's forward outer-reverse inner. In a 0.2-ml PCR-tube made by the English by Biolaps Company, the nucleic acid from each sample is combined with the primer designated for the mutations being studied, together with the components of the master mix, to create the PCR reaction mixture. To guarantee that the components of the reaction are combined, mix in the Microfuge for 5 to 3 seconds. Following that, the PCR tubes were placed into the thermocycler using a unique program for each mutation, the addition of the Ladder DNA prepared by Biolaps Company in one of the first holes, followed by the injection of the reaction product at a concentration of 2% into the pits of the prepared agarose gel. The samples are then migrated using the electrophoresis device for 45 minutes, after which the bands are imaged using a gel-documentation device. Determination of genetic variation of the COLA1 gene at locus ((rs1107946) in using a technique Tetra-ARMS-PCR

Methods

Determination of genetic variation of the COLA1 gene at locus ((rs1107946)) using Tetra–ARMS - PCR technique.

Detection of ((rs1107946)) polymorphism by ARMS-PCR:

The presence of the A C mutation was detected in the site ((rs1107946)), 4 l (100 nanograms) of template DNA and 1 l (10 picompl) of each primer ((rs1107946)) were added. which the researcher created using the Pimer 3 tool, applied to this gene for the first time, and which the Korean business Macrogen manufactured and added to the contents 20 l made up the master mix and the total volume of the reaction.. (Rizk et al., 2019).

Table (1): demonstrates the primers used in the PCR to identify the genetic variant at the locus (rs1107946).

Primer	Sequence	Band size	Annealing
F-outer	GCAGGGGCTATAATTAAAGGGAAA	387bp	59
R-outer	CCCTTCTTACTACGAAAACCCAAGA		
F-inner	ACTGTGGGTCAGTTCCAAGAAAA	258 bp	
R-inner	TGTCGCCTATTAGGGAGGAGG	173bp	

Then the PCR tubes were placed in a thermocycler to conduct the polymerase chain reaction, depending on the special program for the reaction

Table (2): the ARMS-PCR technique to determine the mutation (rs1107946))

No.	Stage	Temperature	Time	Cycle number
	Initial denaturation.	95.0	5.0 min.	1
	Denaturation.	95.0	45.0 sec.	35
	Annealing.	67.0	1.0 min.	
	Extension.	72.0	1.0 min.	
	Final extension.	72.0	7.0 min.	1
	Stop reaction.	4.0	5.0 min	1

Results

Using a method, the COLA1 gene's genetic variant at position ((rs1107946) can be determined.

Tetra-ARMS-PCR

The findings demonstrated, as shown in Figure 1, a connection between women who have osteoporosis and the genetic variation of the COLA1 gene at the site (rs1107946. This is because the PCR reaction's outcome clearly demonstrates that the genetic variation of the gene is present in the three genotypes CC, AC, and AA. And in various percentages as shown in Table (3).

Table (3) Distribution of the allelic incidence and genotype of the COLA1

Genotypes	Patients		Control		P Value	OR
	NO.	%	NO.	%		
CC	44	62	18	90	P = 0.0624	2.9032
CA	20	28	1	5		
AA	6	10	1	5		
Alleles	NO.	%	NO.	%	P Value	OR
C	108	77	37	92	P = 0.0049	3.4351
A	32	23	3	8		

Table (3) shows the allelic incidence and frequency of the different genotypes of the COLA1 gene at the site (rs1107946).

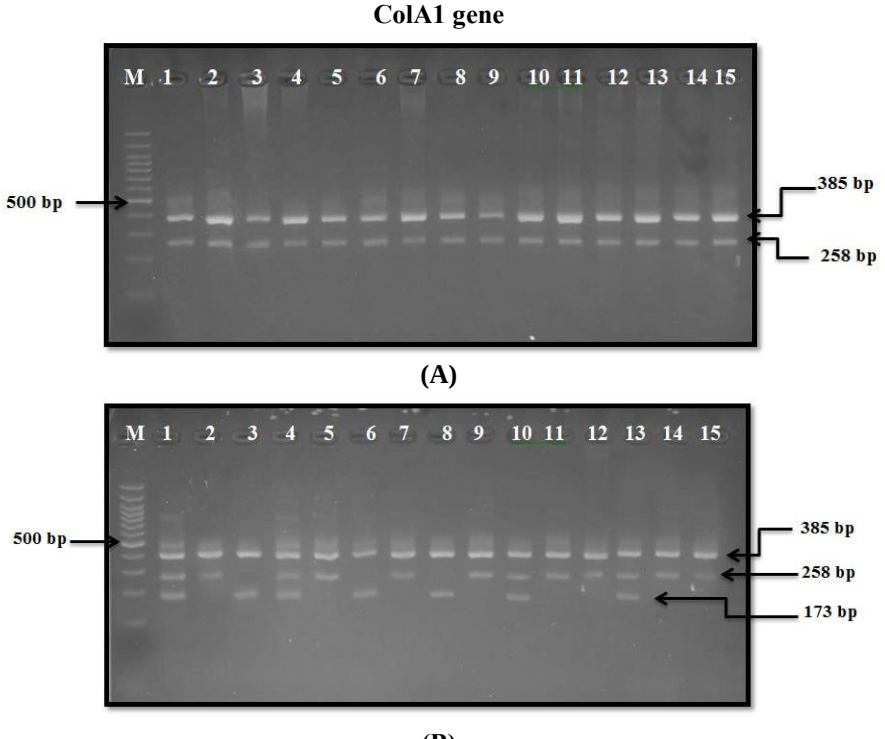

Figure (1) A, B: The product of the PCR reaction for the genetic variation (rs1107946) of the COLA1

In table 3 The frequency value of the AA mutant genotype in the group of women with osteoporosis was 10%, which is the lowest of the genotypes, according to the data for women with osteoporosis. The control group is 5% of the mutant. While the percentage of the normal (normal) genotype AA was largest in the group of osteoporotic women (62% higher than the healthy group, where it was 90%), the heterogeneous genotype AC was lowest in the group of infected women (28%). Compared to the control group (5%), a high number of observations were made.

In terms of allelic recurrence, the findings revealed that the patients' group had a higher incidence of the mutant allele A—23% compared to 8% in the control group. Natural allele prevalence in patients was 62%, as opposed to 92% in the control group.

The study's findings also revealed that the unhealthy genotype (mutant) AA had an odds ratio of 2.90 at the probability level of 0.0278 and the unhealthy allele (mutant) had an odds ratio of 3.43 at the probability level of 0.0135, both of which are above (1) and are regarded as risk factors.

Discussion and Conclusion

One of the gene's causing osteoporosis is the collagen 1 gene, which encodes the 1(I) proteins of the first type, the major protein of bone [15]. genetic variations and mutations affecting this gene affect negatively on the protein encoded by the aforementioned gene and thus the bone structure is affected Also, the occurrence of genetic mutations in the COLA1 gene leads to a defect in the formation of chains and the decoupling of the spiral structure, which leads to weak collagen and thus the occurrence of osteoporosis.[16]

Also, Skeletal abnormalities, which are primarily defined by the development of severe and early osteoporosis, can result from genetic mutations in the gene. Additionally, studies have demonstrated a substantial correlation between clinical variations of the COL1A1 gene's C-terminal propetide and uncommon recessive forms of osteoporosis. [17]

Numerous diseases, most of which are linked to osteoporosis, are caused by more than 400 mutations in this gene, according to earlier research, and a deficiency in this gene results in a functional defect in the genetic regulation of bone mass, susceptibility to fractures, and fragility. [18]

As collagen fibers can act as barriers preventing the infiltration of tumor cells into the cell, studies have shown that the COL1A1 gene is one of the components of the extracellular matrix in some types of cancer, including gliomas. This is because the increase or decrease in collagen deposition of this gene is associated with an increase in malignancy. Understanding how the COL1A1 gene contributes to the formation of gliomas is crucial. [19]

Mutations in the COL1A1 gene (rs1107946 and rs1800012) have been linked to severe musculoskeletal problems such osteogenesis and Ehlers-Danlos syndrome since the COL1A1 gene is the main collagen fibrillary responsible for the function of tendons and ligaments. Studies have revealed alterations in a gene's binding site (rs1800012) that are connected to problems of the musculoskeletal system and soft tissue injuries. [20]

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