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Relation Of Gene And Some Biochemical Parameters In Diabetic Patients With Osteoarthritis

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Abstract

Matrilin-3 is a protein found in the extracellular matrix that is not composed of collagen. The MATN3 gene is found on the short arm of chromosome 2 region 2p24-p23.Matn3 is found at lower levels in mature articular cartilage (AC), but its expression is induced through (OA), This pattern of expression indicates that Matrilin-3 has a role in the etiology of osteoarthritis. Some studies have found that mutations in the MATN3 gene area are a risk factor for OA. this study aimed to examine the association of the Matn3 (rs77245812) SNP(C/T) gene polymorphism as a risk factor for osteoarthritis. genotyping of the SNP(C/T) was performed in 50 type2 diabetes with osteoarthritis, 50 osteoarthritis, and 50 control subjects. Genomic DNA was isolated from venous blood samples, quantified, and subjected to conventional PCR. statistical analysis showed no association found between Matn3 (rs77245812) and the risk developing of osteoarthritis, Genotype frequency distribution (X2= 6.25, P-value= 0.18) allelic frequency distribution (X2= 0.14, P-value = 0.01).

Keywords: Osteoarthritis, Matrilin-3, T2DM, genetic study, PCR.

Introduction

Osteoarthritis is among the reasons that lead to the inability worldwide (1). And is the most commonly complaint of the synovial joints, analogous to the knee, hip, and hands, and the most important source of social costs in aged grown-ups. The World Health Organization (WHO) estimates that by time 2050, 130 million people will have osteoarthritis worldwide and 40 million will be seriously affected. Impaired mobility, pressing the important social burden so serious complaint will represent (2). In fact, OA can impact joint cartilage, synovium, meniscus, muscle, subchondral bone, ligaments, and capsule. This description of OA can be important in characterizing the life of patients because it determines the kind of information to be acquired, such as structural, clinical or surgical (3). The prevalence of the (OA) multiplies with getting older and is much linked to obesity (4), An abstract model for the pathogenesis of OA is that systemic factors (e.g., age, metabolic syndromes, gender, human ethnicity, and genetic factors) increase main capability of

osteoarthritis, while topical mechanical factors (e.g., obese, muscle weakness, and joint trauma or failing) influence its local, and severity. (4). Body fat and obesity which is often remarked in patients with diabetes (DM) may be a significant factor in progress of (OA), especially in the progress of osteoarthritis in the backbones and the lower limbs where a high elevated body weight will increase the load on the joints. The pain may also be a result of osteoporosis which is associated with DM type2 via a vitamin D insufficiency (5). OA and DM type2 extremely live together due to their rise spread and common hazard factors. For i.e., The relation of osteoarthritis with obesity is well propped and obesity happen in most individuals with type2 diabetes (6). the mechanical overload due to the Additional weight, fat cells releasing cytokines into the circulation, causing persistent low-grade inflammation and activating proteolytic enzymes that can cause matrix breakdown and begins osteoarthritis. concurrently, inflammation induced by low-grade fatty tissue affects the metabolic imbalance underlying various metabolic disorders, e.g., type2 DM (7).

DM type2 typical differences in blood sugar, it is believed to elicit similar effects on the glucose concentration in the synovial fluid of the cartilages and, therefore, also influences the glucose equipping of joints chondrocytes (8). The heritability of OA is about 50% and former genetic studies have characterized (twenty-one) loci in total, traverse hip, hand, and knee OA with limited interfere (9). mutations and Polymorphism in some genes, like Transforming Growth Factor Beta (TGF-β), Bone Morphogenetic Protein (BMP), Frizzled-Related Protein 3 (FRP3), are secreted (FRZB), Growth Differentiation Factor 5 (GDF5), and matrilin3 leads to early OA in individuals (10). Therefore, this study focuses on investigating the association of gene polymorphism of matrilin 3 and the risk of having diabetes mellitus type 2 with osteoarthritis in both sexes, and evaluate the association between gene polymorphism of the MATRILIN_3(RS77245812) and evidence of osteoarthritis. the mechanical overload due to the Additional weight, fat cells releasing cytokines into the circulation, causing persistent low-grade inflammation and activating proteolytic enzymes that can cause matrix breakdown and begins osteoarthritis (5). The heritability of OA is about 50% and former genetic studies have characterized (twenty-one) loci in total, traverse hip, hand, and knee OA with limited interfere (6). mutations and Polymorphism in some genes, like Transforming Growth Factor Beta (TGF-β), Bone Morphogenetic Protein (BMP), Frizzled-Related Protein 3 (FRP3), are secreted (FRZB), Growth Differentiation Factor 5 (GDF5), and matrilin3 leads to early OA in individuals (7). Therefore, this study focuses on investigating the association of gene polymorphism of matriline 3 and the risk of having osteoarthritis in both sexes, and evaluate the association between gene polymorphism of the MATRILIN_3(RS77245812) and evidence of osteoarthritis.

Materials and Methods

Samples were collected from (150) Iraqi southern patients ranging in age from 40-60 and They were separated into 3 groups, each group as follows:

- Group a: (50) (DM) with (OA) patients, 27 females and 23 males.
- Group b: (50) patients suffering from osteoarthritis,36 females and 14 males.
- Group c: (50) participants who were not diabetes and did not have osteoarthritis (control group), 18 females and 32 males.

A two ml of blood samples in the EDTA tube was stored frozen at -20°C for DNA isolation to identify a single nucleotide polymorphism (SNP) of MATRILIN-3 (RS77245812).

Anthropometric Evaluation

A scale on the wall was used to measure the height (cm), and a digital weighing machine was used to measure weight (kg). Subjects' body mass index (BMI) was estimated using the equations = weight (Kg) / height (m2).

Primers Design

Specific two primers that were used for discovering a specific sequence of SNP (rs77245812) which designed by Fast PCR and provided by IDT(Canada).as shown in table (1).

Table 1: Primers used in this study

SNP		Sequence (5'-3')	Allele	TM*	PCR product Size (bp)	Source
MATN-3 (RS77245812)	F1	**5-** CCTTGAATGCC GACAAGAAAA**C**-3	C	59-60° C	202 bp	NCBI
	F2	**5-** CCTTGAATGCC GACAAGAAAA**T**-3	T	59-60° C	202 bp	
	R	5- CTTTTCTCCCTAAGCTCCTTTTCAG-3		59-60° C	202 bp	

Experimental design

Genomic DNA was Isolated by the Easy Pure® Blood Genomic DNA Kit, and the nanodrop spectrophotometer instrument (THERMO. USA) was used to assess the extracted DNA concentration (Nano gram/microliter) and purity, taking absorbance readings from 260 -280 nanometers. and the pure DNA was stored at -20 Co. Prepare an agarose gel (1.5 %) concentration was used after conventional PCR product detection (Table 2.), The DNA has been seen by staining the gel with ethidium bromide and observing it via a UV transilluminator.

Table 2: PCR amplification program

SNP	Initial Denaturation	Cycling conditions			Final Extension	Hold	Cycle No.
		Denaturation	Annealing	Extension			
rs77245812-MATN3	94°C/180sec	94°C/30sec	53.5° C/ 30 sec	72°C/30sec	72°C/600sec	4°c	35

Results and Discussion

Our study result with clarity showed significant difference in BMI mean levels in (DM+OA) and OA patients' groups as compared to controls (30.63 ± 2.56a, 30.70 ± 3.74a, and 25.59 ± 0.58b) respectively, (L.S.D = 1.04). (Table 3.).

Table 3: BMI of study subjects

	Control Subjects	DM+ OA	OA	
NO. (M, F)	50(32/18)	50(23/27)	50(14/36)	0.01 P-value
BMI (kg/m^2)	25.59 ± 0.58^b	30.63 ± 2.56^a	30.70 ± 3.74^a	1.04 L.S. D

* Means having different letters in the same column differed significantly (P≤0.05). (L.S.D) least significant difference.

Polymorphism Genotype

Results of amplification reactions (conventional PCR)

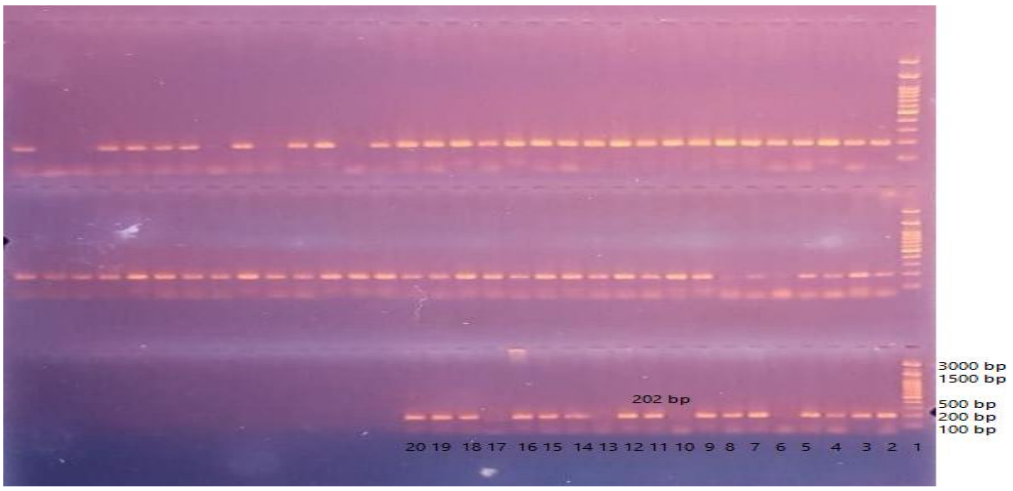

Figure 1: PCR amplification of matrilin-3 rs (77245812) using specific primers for detection of the Allele C was electrophoresed on 1.5% agarose gel. Lane 1 represents a 100 bp DNA ladder. Lanes (2-20) represent PCR products (202 bp) from different patients.

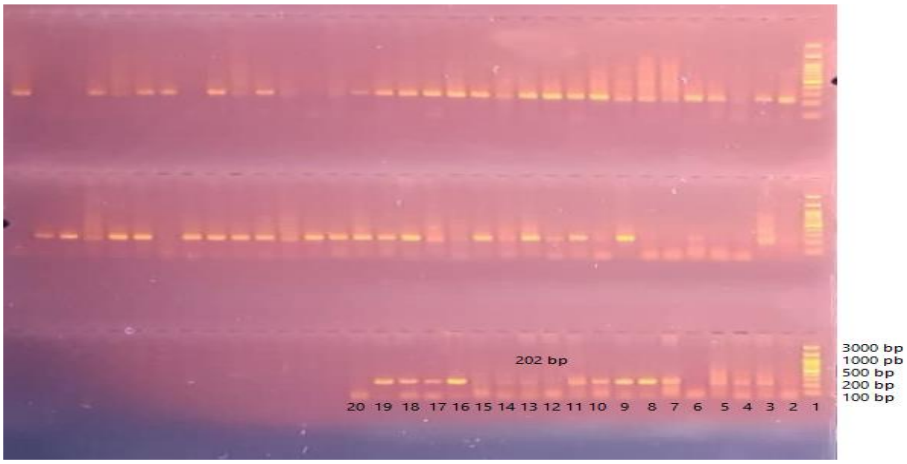

Figure 2: PCR amplification of matrilin-3 rs (77245812) using specific primers for detection of the Allele T was electrophoresed on 1.5% agarose gel. Lane 1 represents a 100 bp DNA ladder. Lanes (2-20) represent PCR products (202 bp) from different patients.

Genotype and Allele Frequency

The genotype distribution of matrilin-3 rs (77245812) T/C SNP in (DM+ OA), OA patients and control subjects found no significant difference (P- value = 0.18), While the results of the allele frequency distribution of matrilin-3 rs (77245812) C/T SNP found statistical analysis showed significant difference (P-value = 0.01) among DM+ OA, OA and control groups (Table 3.) and (Figure 3.)

Table 4: Genotypes and allele frequencies of MATN-3 rs (77245812) gene polymorphism in patient's groups, and control subjects.

Genotype	DM + OA	OA	Control
CT	46 (92%)	41 (82%)	42 (88%)
CC	2 (4%)	7 (14%)	4 (8%)
TT	2 (4%)	2 (4%)	2 (4%)
Total	50 (100%)	50 (100%)	48 (100%)
		X^2 = 6. 25 df = 4 P- value = 0.18	
Allele	**DM + OA**	**OA**	**Control**
C	48 (50%)	48 (55%)	46 (58%)
T	48 (50%)	43 (45%)	44 (42%)
Total	96 (100%)	91(100%)	90 (100%)
		X^2 = 0.14 df = 2 P- value = 0.01	

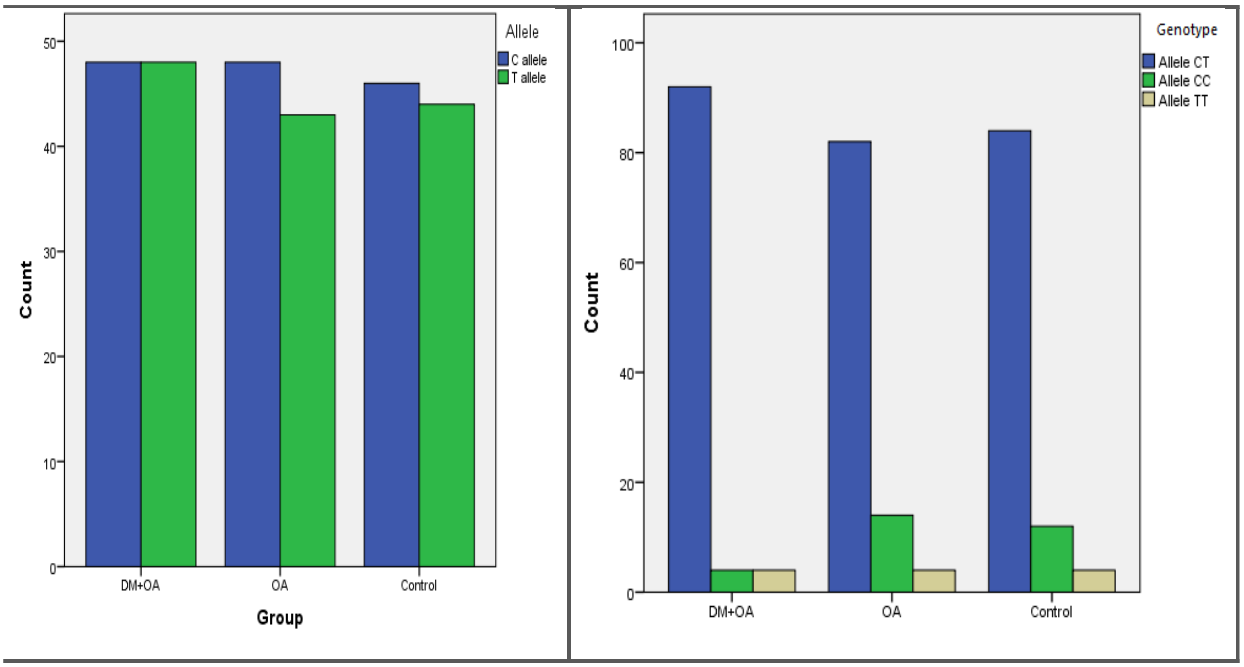

Figure 3: distribution of MATN-3 rs (77245812) C\T SNP genotypes and the Allele among patient's groups and control subjects.

Gender Distribution Within Genotypes in Patients and Control Subjects

the genetic distributions of matrilin-3 rs (77245812) C/T SNP in males 59 had the CT, 6 had the CC, and 5 had the TT genotype. while in females 70 had the CT, 7 had the CC, and 1 had the TT genotype in DM+OA, OA, and controls in respectively. were shown in (table 4.) statistical analysis showed a significant difference (P-value = 0.01).

Table 5: Gender distribution of MATN-3 rs (77245812) SNP genotypes in patient groups and control subjects.

Groups	CT	CC	TT	Total
DM+ OA	M 22 (44.0%) F 24 (48.0%)	M 1(2.0%) F 1 (2.0%)	M2 (4.0%) F0 (0.0%)	25 25
OA	M 11 (22.0%) F 30 (60.0%)	M1 (2.0%) F 6 (12.0%)	M1 (2.0%) F1 (2.0%)	13 36
CON.	M 26 (54.2%) F 16 (33.3%)	M4 (8.3%) F 0 (0.0%)	M2 (4.2%) F0 (0.0%)	32 16
Total	M = 59 F = 70	M = 6 F = 7	M = 5 F = 1	M =70 F = 78
X2 = 24.61 df = 10 P-value =0.01				

Discussion

The most prevalent cause of joint deterioration, pain, and disability is osteoarthritis (11). Almost half of T2DM patients (47.3%) have some kind of arthritis (6), Furthermore, (12) discovered that the risk of OA in diabetes individuals was greater than in non-diabetic patients, even when only studies adjusting for weight or BMI were included. The goal of this study was to investigate the MATN-3 (rs77245812) gene polymorphism in diabetic patients suffering from osteoarthritis with an emphasis related to clinical parameters. the MATN3 gene, which may be found on chromosome 2 little arm in the 2p24p23 region (13). in our study, no association was found between MATN-3 (rs77245812) gene polymorphism and OA in the southern Iraqi population, the distribution frequency of MATN3(rs77245812) SNP genotypes CT, CC, and TT in (DM+ OA) were 92%, 2%, 2%. in OA group were 82%, 14%, 4%. and in control subjects were 88%, 8%, 4% chi- square test showed there was no statistically different in genotypic frequency (X2 = 6.25, P- value 0.18). frequency distribution of C allele and T allele in (DM+OA) were 48% and 48%, in OA group were 48% and 43%, and in control subjects were 46% and 44% chi- square test showed statistically different in allelic frequency (X2 = 0.14, P- value 0.01).

Several association studies between single nucleotide polymorphisms (SNPs) and OA remain controversial due to bias in the inclusion criteria of patients with OA, that is, different sites of the joint affected by the disease, different classification, and stratification designs, the variability of the scales used in the radiographic evaluation and subjective scores in patient pain assessment. Furthermore, investigating the ethnic and regional allelic distribution, which is critical for completely understanding the impacts of genetic variations (14). these our statistics analysis results show that OA may be linked to other potential factors, Given the complexity etiology and physiopathology of illness as well as genetic variation. in a study by Minafra et al. (2014), there is no link between MATN3 (rs77245812) gene polymorphisms and the risk of knee OA (15). Sydorchuk et al. (2017), polymorphic variations of the gene MATN3 (rs77245812) are not a risk factor for the emergence of OA in the Northern Bukovina population (16). And according to García -Alvarado et al. (2020), there is no link between MATN3 (rs77245812) gene polymorphisms and the risk of getting KOA among

Mexican mestizos (14). Although the significance of MATN3 in the risk of OA is unclear, several studies have found that changes in the MATN3 gene area are an important risk factor for OA (14). Like Gu et al. (2012) discovered a link between MATN3 gene polymorphism and KOA in the Chinese Han population (17). Furthermore, according to Diab et al. (2017) the MATN3 gene may be associated with the incidence and severity of knee OA in Egyptian patients. This may indicate a connection to ethnic and regional diversity (18).

BMI

Recent research has found a link between BMI and type 2 diabetes. These effects are due to an increase in the production of proinflammatory mediators from adipocytes in obese people, such as (TNF- α), interleukin (IL)-6, and IL-1. These factors have been linked to systemic low-grade inflammation in people with both OA and T2D (19). Several studies have found that those with a BMI of 30 or above are more probable to develop radiographic and clinical OA (20). Obesity is one of the primary risk factors for knee OA, and there have been findings linking it to hand OA as well. However, the data for hip OA is more ambiguous. Both mechanical loading of the weight-bearing joint and activation of metabolic variables contributing to joint deterioration have been hypothesized as plausible pathways to explain how obesity raises the risk of knee or hand OA (21).

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