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The Relationship Between Col1a1 Sp1 Binding Site Polymorphism and Musculoskeletal Disorders in Type 2 Diabetes Mellitus

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Abstract

Aim: In our study, we aimed to identify the musculoskeletal disorders in patients with type 2 diabetes mellitus (DM) and to show the possible relationship with COL1A1 Sp1 binding site polymorphism.
Methods: 75 patients with type 2 DM applied to the study aged between 18 and 65 years old.75 volunteers were enrolled in the study as a control group. The musculoskeletal examination of the patients and the control groups were performed by a single physician. All samples from the patient and control groups were assessed in the molecular genetics diagnostic laboratory. The polymorphism of COL1A1 gene was studied by PCR-RFLP method.
Results: The statistically significant differences between the groups were as follows: adhesive capsulitis (AC) (p=0.002), Dupuytren's contracture (DC) (p=0.043), limited joint mobility syndrome (LJMS) (p=0.000) and carpal tunnel syndrome (CTS) (p=0.003). Among the patients of the study group, the clinical parameters that were significantly associated with complications were as follows: HbA1c levels (p=0.016) for AC, diabetes duration (p=0.019) for DC, BMI (p=0.014) for LJMS and sex (p=0.000) for CTS. While the difference in the prevalence of 'GT'genotype was significant (p=0.004) and having a high'T' allele may be associated with an increased risk musculoskeletal complications in the patients (p=0.019).
Conclusion: Our results support the view that the COL1A1 gene polymorphism is associated with an increased risk of musculoskeletal complications in diabetic patients whose having the "GT" genotype or higher levels "T" allele expressed.
Keywords: COL1A1 gene polymorphism, musculoskeletal disorders, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a disease including a group of metabolic disorders characterized by hyperglycemia. If hyperglycemia cannot be controlled, it may cause a variety of functional derangements and impairments in organs (1). Complications of DM can be acute or chronic when we consider temporal relation, and can be local or systemic depending on the extent of disease (2). It is well known that musculoskeletal

complications are relatively frequent and directly effecting the life quality of patients. Musculoskeletal complications of DM are summarized in Table 1 (3).

Collagen is the structural component of extracellular matrix and classified according to the functional characteristics. The most abundant type of collagen in extracellular matrix is type 1 collagen (4). Type 1 collagen fibrils are composed of two alpha-1 and one alpha-2 chain (5, 6). Alpha-1 chain is coded by COL1A1, and alpha-2 chain is by COL1A2 (7-10]. COL1A1 gene is located on chromosome 17p21.2-22 and contains 52 exons and 51 introns (11). Sp1 as a binding region plays important roles in the regulation of gene expression (12). Any potential nucleotide polymorphisms at the site of Sp1 binding region can increase the expression rate up to three times (5). Excess amount of alpha-1 collagen chains, together with alpha 2 collagen, can cause the formation of an abnormal homotrimeric collagen (13). Even though COL1A1 gene polymorphism is studied in context of several diseases, there is only minority of studies in DM.

The aim this study was to define the musculoskeletal disorders in patients with type 2 DM and investigate the relationship between Col1a1 Sp1 binding site polymorphism and these complications.

Table 1. Musculoskeletal complications in diabetes mellitus

Adhesive capsulitis
Dupuytren contracture
Limited joint mobility syndrome
Flexor tenosynovitis (trigger finger)
Carpal tunnel syndrome
Diabetic osteoarthropathy (Charcot arthropathy)
Diffuse idiopathic skeletal hyperostosis (DISH)
Diabetic muscle infarction
Crystal-induced arthritis
Reflex sympathetic dystrophy

Materials and Methods

75 patients with type 2 DM as per WHO criteria, between the ages of 18 and 65 with no sex preference were included to the study (14). 75 healthy control were constituted from the age-matched non-diabetic people from the same region of the country. The exclusion criteria were thyroid diseases, pulmonary tuberculosis or cancer, history of stroke, myocardial infarction, shoulder trauma or surgical intervention. The local ethics committee reviewed and approved the study, and informed consent was obtained for all subjects. The research was in compliance with the Declaration of Helsinki.

Musculoskeletal examination for both groups was done by the same physician. For the evaluation of adhesive capsulitis (AC), diagnostic criteria were used. These criteria were shoulder pain with one month of duration, awakening from the sleep, not leaning on the affected shoulder, and

movement restriction for three planes (15). Most frequently affected shoulder movements (external rotation, abduction and internal rotation) were evaluated. Although the radiographic investigation was normal in patients with AC except the mild osteopenia due to prolonged resting, it was used in order to exclude the other reasons including the tumor or arthropathy. Diagnosis of dupuytren's contracture (DC) was made according to presence of palmar or digital nodule, palmar or digital skin thickening, a pretendinous band and one or more digital contracture (16-18).

Presence of limited joint mobility syndrome (LJMS) was evaluated by prayer sign test. With the wrists flexed, patients were asked to unite their palmar aspects of the hands and the test was considered positive if the patient could not unite the metacarpophalangeal and proximal interphalangeal joints (16,17).

Carpal tunnel syndrome (CTS) was diagnosed with pain and paresthesia of first, second and third fingers, and Tinel and Phalen tests. Diagnosis was also supported with electromyography (16).

Genetic Analysis

Samples from the patients and healthy group are evaluated in molecular genetics laboratory. COL1A1 gene polymorphism is detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). 2 ml peripheral blood was taken from each patient and control group into tubes with EDTA, and then DNAs are isolated from the leukocytes with isolation kits. 0.2 mM dCTP, dATP, dGTP and dTTP, 1.5 mmol MgCl2, 0.4 µM from each primer, 0.35 U Taq DNA polymerase and 35 ng DNA were placed in PCR medium. RFLP analyses of these PCR products were used with the aid of Msc1 restriction enzyme. RFLP products are placed in an 3% agarose gel and photos are taken with gel imaging system.

Statistical Analysis

Normality assumption was checked by using Kolmogorov Smirnov test. To compare the two groups, student's t tests for normally distributed continuous variables and Mann Whitney U test for non-normal variables, and chi-squared tests for categorical variables were used. Genotype frequency distribution was determined by Hardy-Weinberg equilibrium. $P<0.05$ was accepted as significant.

Results

Mean age for patients and healthy controls were 51.72±7.7 and 49.97±8.8 years respectively. Patient group consisted of 46 females and 29 males, control group included 50 females and 25 males. There was no statistically significant difference for demographic variables between both groups ($p>0.05$). Average body mass index (BMI) was 30.98±4.5 kg/m^2 and 26.59±3.9 kg/m^2 in patient and control group respectively. Mean duration of DM was 4.09±5.6 years, and mean HbA1c level was 7.73%±2.2.

AC, DC, LJMS and CTS numbers between patient and control group were statistically significant ($p<0.05$). Findings of musculoskeletal examination for both groups were summarized in Table 2.

Table 2. *Musculoskeletal findings in patient and control gorup*

Symptoms	Patient group (%)	Healthy control (%)	p value
AC	14 (18.6)	2 (2,6)	0.002
DC	4 (5.3)	0 (0)	0.043
LJMS	43 (57.3)	18 (24)	0.000
CTS	34 (45.3)	17 (22.6)	0.003

AC, adhesive capsulitis; DC, Dupuytren contracture; LJMS, limited joint mobility syndrome; CTS, carpal tunnel syndrome

Relationship between AC, DC, LJMS and CTS and duration of DM, age, gender, BMI and HbA1c in diabetic patients was summarized in Table 3.

Table 3. *The relationship between musculoskeletal complications and clinical parameters*

Complications	Clinical parameters	p value
AC	HbA1c	0.016
DC	Duration of diabetes	0.019
LJMS	BMI	0.014
CTS	Gender	0.000

AC, adhesive capsulitis; DC, Dupuytren contracture; LJMS, limited joint mobility syndrome; CTS, carpal tunnel syndrome

Genotype and alleles of COL1A1 in both groups were summarized in Table 4. When we compare the alleles and genotype distribution in both groups, existence of a deviation in patient group was supported by Hardy Weinberg Equilibrium. Also, GT genotype and T allele is statistically much more frequent in patient group.

Table 4. *COLLIA1 Gene Sp1 binding site polymorphisms (Chi-square test)*

COL1A1 gene Genotypes	Healthy control (%)	Patients (%)	P value
GG(SS)	65 (86)	51(68)	0.377
GT (Ss)	9(12)	24 (32)	**0.004**
TT (ss)	1 (2)	0	0.377
GG+GT	74 (98)	75 (100)	0.315
GT + TT	10(13)	24 (32)	**0.006**
Total	75	75	
Alleles			
G	139	126	**0.019**
T	11 (7)	24(16)	
Total	150	150	
HWE (p)*	0.379	0.032	

*HWE :Hardy-Weinberg Equilibrium

Discussion

Our results demonstrated that musculoskeletal disorders were more common in patients with type 2 DM and related with some personal and clinical factors and also showed that Col1a1Sp1 binding site polymorphism, namely GT genotype, and patients with higher T allele frequency might be associated with these complications.

Musculoskeletal problems as one of the long term complications of DM impair the quality of life (19). The relationship between the DM and AC has been investigated in several studies. Prevalence of AC was 7-32% in patients with type 2 DM, and 0-10% in control groups (15,20,21). In our study, 18.6% of patients with type 2 DM and 2.6% of control group had AC. In a study investigating the relationship between AC and duration of DM, age, gender, and HbA1c levels, a statistically significant relationship was found between the duration of disease and age, but gender and HbA1c levels had not statistically significant influence (21). In another study, a significant relationship between AC and age was detected, but gender, duration of DM, BMI and HbA1c levels were not related significantly (15). Our study had showed that there was a significant correlation between AC and HbA1c levels, but duration of DM, gender, age and BMI had no significant influence. Cause of not finding a relationship with duration of DM can be explained by the low number of patients. Also, significant relationship between HbA1c and AC may be attributed to the poor glucose control and its consequence.

Trap neuropathies as a risk factor for CTS are frequently encountered symptoms in DM. CTS can be seen in up to 15% of general population and up to 25% in diabetics (22,23). In our study, 45.3% of patients and 22.6% of control group had CTS. High prevalence in control group may be explained by the other factors causing carpal tunnel syndrome including small diameter of canal, obesity and frequent use of wrist.

In studies investigating the relationship betwen DM and DC, DC was found 12-14% of diabetics and 3% of healthy controls (20,24,25). 5.3% of type 2 diabetics of our study and none of the patients in healthy control group had DC. Low percentage of DC in our study can be explained by the low number of patients. Studies investigating the relationship between DC and duration of DM, age, HbA1c level showed a direct correlation between DC and duration of DM and no relation between age and HbA1c levels as in our study (20,26). Similar to other studies, we demonstrated a significant correlation between DC and duration of diabetes, however there was no significant relationship between disease duration and age, gender, BMI, and HbA1c levels.

LJMS, which is also known as diabetic cheiroarthropathy and stiff hand syndrome, was demonstrated in 25-76% of type 2 diabetics and 1-20% of healthy people (17,27-30). In this study, LJMS was the most frequent musculoskelatal problem of diabetics with a frequency of 57.3% in diabetics, and 24% in control group. Studies demonstrated that LJMS was correlated with the duration of diabetes and the age of patient (27,29,31), but no significant relationship was found between LJMS and gender, HbA1c and BMI (28,32). In this study, there was a significant relationship between LJMS and BMI, but no such relationship was found for the duration of diabetes, age, gender, and HbA1c levels. The lack of any significant relationship can be attributed to the low number of patients included in this

study who had lower mean age with disease duration less than 10 years in most cases.

Collagen type 1 constitutes 80% of the protein in bone tissue. Any change in alpha1/alpha2 chain ratio may result in increased bone fragility and a significantly higher risk of osteoporosis (33-35). Patients with GT genotype were found to have less inorganic, but higher organic component in bone tissue which impairs mineralization and strength of the bone (36). In addition, non-athletic patients were found to have higher risk of anterior cruciate ligament rupture (37). Patients with achiles tendinopathy had higher type1 and type3 collagen mRNA levels (38). COL1A1 gene expression was higher in patients with painful achiles tendinopathy (39). Other studies found a significant relationship between otosclerosis and COL1A1 gene polymorphism (5). COL1A1 gene was found to be significantly active in patients with diabetic nephropathy, cardiomyopathy, arteriosclerosis, and aortic fibrosis (40-43), but to our knowledge, this is the first study to show COL1A1 gene activity in diabetic patients with musculoskeletal complications. Our results support that patients with GT genotype and higher T allele frequency carry higher risk of developing musculoskeletal problems. We beleive that diabetic patients with this genotype and/or the ones with higher T allele frequency have higher risk of developing musculoskeletal complications. Physicians should be careful for musculoskeletal complications in these patients and efforts should be made to decrease the associated morbidity with earlier rehabilitation programs.

Conclusion

In conclusion, diabetic patients, having a particular COL1A1 Sp1 binding site polymorphism, namely GT genotype, and patients with higher T allele frequency were found to have higher frequency of musculokeletal complications. Despite the difficulty in classification of musculoskeletal disorders in diabetic patients, further studies are required to investigate the role of COL1A1 gene polymorphisms in subclasses of these complications.

Conflict Of İnterest: None

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