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HFE Gene Mutation Among Turkish Patients with Type 2 Diabetes Mellitus

Türk Tip 2 Diabetik Hastalar Arasında HFE Gen Mutasyonu

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Abstract

Purpose: Hereditary haemochromatosis (HH) is a genetic disease with autosomal recessive trait. Recent studies demonstrated the importance of C282Y gene mutation in the aetiology of HH. Free iron accumulating in pancreas deteriorates insulin secretion and synthesis which can lead to insulin resistance and the development of type 2 diabetes mellitus (T2DM) in patients with HH. There has been no study determining the prevalence of haemochromatosis gene (HFE) mutations and HH in diabetic patients in Turkey. We planned this study in order to investigate the C282Y and H63D mutation that cause HH in T2DM.

Material and Method: In this study, we included185 patients with T2DM. Patients older than thirty-five years, not taking vitamin supplementation, iron preparates and/or oral contraceptives and those without any signs of active bleeding were included while patients with any infectious, systemic or immune disease were excluded from the study. Serum transferrin saturation (TS), ferritin, iron, and total iron binding capacity levels were measured after 12 hours of fasting.

Results: Ten (5.4%) cases with TS of more than 45% were detected at the first evaluation. The test was repeated in those cases and 6 patients with TS of more than 45% were left according to the second measurement. H63D and C282Y gene polymorphisms were not present in these patients.

Discussion: We did not find any correlation between the existence of T2DM and HFE polymorphisms. We assume that screening for HH in T2DM in our population is not needed. *Turk Jem 2013; 17: 89-91*

Key words: Hemochromatosis, Type 2 diabetes, transferrin saturation

Özet

Amaç: Herediter Hemokramotozis (HH) otozomal resesif özelliğinde genetik bir hastalıktır. Önceki çalışmalar,HH etyolojisi için C282Y gen mutasyonunun önemini göstermiştir. HH'lu hastalarda pankreasta serbest demir birikmesi, insülin sekresyonunu ve sentezini bozarak, insülin resistansı ve tip 2 diabet mellitüs (DM) gelişimine öncelik etmektedir. Türkiyedeki diabetik hastalarda HFE gen mutasyonu ve HH prevalansı belirlemeye yönelik herhangi bir çalışma bulunmamaktadır. Biz bu çalışmada, tip 2 DM de HH sebep olan C282Y ve H63D mutasyonunu araştırmayı planladık.

Gereç ve Yöntem: Bizim çalışmamıza tip 2 DM tanısı olan 185 hasta dahil edildi. Hastalar 35 yaşın üzerinde,vitamin desteği ,demir preparatı ve/veya oral kontraseptif tedavi almayan ve aktif kanama işareti olmayan hastalar dahil edilirken, infeksiyon, sistemik ve immün sistem hastalığı bulunanlar çalışma dışında bırakıldı. 12 saat açlık sonrası serum transferrin saturasyonu (TS), ferritin, demir ve demir bağlama kapasitesi ölçüldü.

Bulgular: İlk değerlendirmede 10 vakada (%5.4) transferrin saturasyonunun % 45' in üzerinde bulundu. Bu vakalarla test tekrarlandı ve ikinci değerlendirmede 6 vakada TS %45 'in üzerinde bulundu. Bu hastaların hiçbirinde H63D ve C282Y gen polimorfizmi saptanmadı.

Tartışma: Biz tip 2DM ve HFE poliformizmi arasında bir korelasyon bulamadık. Bizim toplumumuzda tip 2 DM li hastalarda, HH için mutasyon taramayı önermiyoruz. *Turk Jem 2013; 17: 89-91*

Anahtar kelimeler: Hemokromatozis, Tip 2 diabetes mellitüs, transferrin saturasyonu

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Introduction

Hereditary haemochromatosis (HH) is a genetic disorder characterized by increased dietary iron absoption and accumulation in body tissues such as liver, pancreas, joints and pituitary gland. Iron accumulation in body tissues may lead to cirrhosis, hypogonadism and arthralgia (1). Even though diabetes mellitus is a well-known complication of HH, the percentage of people presenting with typical type 2 diabetes mellitus (T2DM) having polymorphisms in the HFE gene is not exactly known (2). The HFE gene product is a HLA-like molecule which is presented at cell surface. It is bound to ß2-microglobulin, where it is proposed to modify the affinity of transferrin to its receptor. Although C282Y gene predicts serum iron indices (3-5), reports of C282Y allele frequency in T2DM are conflicting (6-8).

The C282Y mutation in the HFE gene is the main one which causes haemochromatosis. 83% of hemochromatosis patients are homozygous for this mutation (9). The H63D polymorphism, which is the second variant of HFE gene, is not per se associated with hemochromatosis, but it acts synergistically with C282Y mutation (9). Body iron stores are shown to be associated with abnormal glucose tolerance and insulin resistance (10). Nonetheless, the results are open to discussion. Many studies tried to affirm the hypothesis that heterozygosity for hereditary hemochromatosis-causing mutations could be a risk factor for diabetes (10). Kwan et al. found an increased frequency of C282Y mutations in patients with T2DM and they stated that the C282Y gene mutation was a potential genetic marker for T2DM (11). In a study by Fernandes-Real et al., the C282Y allele frequency was similar in patients with T2DM and controls, however, the H63D allele frequency was significantly increased in patients with T2DM. Many studies failed to show this association (12). The prevalence of C282Y and H63D mutations was not found to be increased in patients with T2DM compared to non-diabetic population in some other studies (13-16). Thus, we investigated the association of the C282Y and H63D mutations with T2DM in the Turkish population.

Materials and Methods

One hundred eighty-five patients (110 female and 75 male) with T2DM diagnosed according to the ADA criteria were included in this study. Patients older than thirty-five years, not using vitamin supplements, iron preparates or oral contraceptives, and with no active bleeding were included. Those with any infectious, systemic or immune disease were excluded from the study. Serum transferrin saturation (TS), ferritin, iron, and total iron binding capacity were evaluated after 12 hours of fasting. The test was repeated in subjects whose serum TS levels were more than 45%. TS levels of more than 45% were detected in 6 patients. H63D and C282Y mutations were analyzed in these patients. Serum iron levels and serum total iron binding capacity were measured using an automatic spectrophotometric analyzer. Serum ferritin levels were measured by immunometric assays. TS% was calculated by dividing serum iron levels by total serum iron binding capacity multiplied by 100%. DNA was extracted from EDTA-anticoagulated blood samples collected from the patients using standard methods. HFE C282Y genotyping was performed by the PCR-RFLP method using modified oligonucleotide primers sequence to improve allelic discrimination (5'-CTA CCA GGG CTG GAT AAC CTT G, and, 5'-TGG CTC TCA TCA GTC ACA TAC C) (17). H63D genotyping was performed similarly as described above. All patients were Turkish and they participated in the study after signing an informed consent form. The study protocol was approved by the Ethics Committee of the Ministry of Health Dışkapı Y.B. Education and Research Hospital.

Results

The average age of the patients was 57.3±10.1 years and the duration of T2DM was 11.2±7.2 years. Serum TS levels of more than 45% were detected in 10 patients (5.4%). The test was repeated in these patients and 6 patients were found to have the same results. These patients were analyzed for H63D and C282Y gene polymorphisms, but none of the mutations were detected (Table 1).

Discussion

The C282Y mutation in the HFE gene may lead to haemochromatosis. Furthermore, 83% of hemochromatosis patients are YY homozygotes (9). The H63D polymorphism is the second variant of HFE gene and acts synergistically with C282Y mutation. (9). The relationship between accretion of iron and increased risk of type 2 diabetes had been attributed to insulin synthesis and secretion inhibitory effect of iron (18-19). Increased body iron may cause oxidation of free fatty acids that bring out free radicals (20). Increased free fatty acid oxidation diminishes glucose utilization in muscle tissue and increases gluconeogenesis in the liver, leading to insulin resistance (18-20). Studies on non-cirrhotic haemochromatosis and on hypertransfused patients with

Table 1. H63D and C282Y mutations of the patients (Transferrin saturation > 45%)

Age (year)	Ferritin (ng/ml)	Iron (µg/dl)	Iron binding capacity (µg/dl)	TS (%)	H63D mutation	C282Y mutation
65	357	120	235	51	negative	Negative
76	261	134	194	45.5	negative	Negative
65	187	154	311	49,5	negative	Negative
69	135	151	331	45.6	negative	Negative
42	110	113	229	49,3	negative	Negative
55	355	161	307	52,4	negative	Negative

ß-thalassemia support the evidence that accumulation of iron leads to development of insulin resistance (21-22). Clarke et al. studied the prevalence of HFE gene mutation in a limited number of Turkish people and found that the H63D allele frequency was 17.7% while C282Y mutation was not present (23). Recently, 4633 subjects (3827 men, 806 women with a mean age of 35±8.0) were investigated for hereditary haemochromatosis in terms of C282Y and H63D mutations in a study by Barut et al. (24) and 11 (10 men, 1 woman) subjects were found to be heterozygous while 1 case was homozygous for the H63D mutation, however, the C282Y mutation was not detected in any of them. Simsek and collegues (25), has found H63D mutation in 60 out of 143 HH patients in whom 49 of them were heterozygous and 11 of them were homozygous for this mutation. They also declared that none of their patients had C282Y mutation.

We did not detect C282Y mutation in our study population consistent with the results above, but the absence of H63D can be attributed to the insufficient number of subjects involved in our study.

One study reported the increased prevalence of HFE mutation in diabetic patients compared to control subjects and also indicated that the C282Y mutation was more common among patients with diabetic nephropathy when compared with normoalbuminuric ones (26). However, our results were similar to the results of other studies which did not show increased prevalence of C282Y and H63D mutation among diabetic patients. Routine screening for HH in diabetic patients is not recommended but HH can be suspected as an aetiologic factor in patients who required early insulin therapy. Genetic analyses must be performed for selected patients in our population after phenotypic scanning. Patients susceptible to haemochromatosis with elevated serum ferritin levels and TS can undergo genetic analyses earlier.

The lack of a control group is the main limitation of this study. We recommend genetic analyses for HFE gene mutation in diabetic patients who have clinical, biochemical and phenotypical characteristics of HH. Further studies with larger sample size are needed to investigate the prevalence and importance of HFE gene mutation in the Turkish population with diabetes.

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