The prevalence of primary hereditary hemochromatosis in central Anatolia

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Background/aims: Hereditary hemochromatosis is an autosomal recessive disorder associated with the HFE genes. Early identification and diagnosis is important as end stage organ damage may occur if treatment is delayed. This study aimed to identify the prevalence of hereditary hemochromatosis in Kayseri and surroundings known as Central Anatolia. Materials and Methods: 2304 participants (1220 males, 1084 females) who were older then the age of 17 were included in the study conducted between December 2005 and December 2006 in Kayseri, Turkey. Transferin saturation was measured from overnight fasting blood samples. Serum iron, total iron binding capacity, and transferrin saturation were measured. Serum ferritin levels and hereditary hemochromatosis genetic analysis were also performed after an overnight fasting blood samples from participants whose transferin saturation results were more than 50% in man and more than 45% in women. Results: The homozygote C282Y mutation and heterozygote C282Y mutation prevalences were found as 0.08% (1/1220) and 0.08% (1/1220) in male participants, respectively. The heterozygote H63D mutation prevalence was found in 0.09% (1/1084) of female participants. Calculated prevalences in general population are as follows: The homozygote C282Y mutation prevalence is 0.043% (1/2304), the heterozygote C282Y mutation prevalence is 0.043% (1/2304) and the heterozygote H63D mutation prevalence is 0.043% (1/2304). Conclusions: The prevalence of hereditary hemochromatosis in Central Anatolia is 0.043% (1/2304). Because of the relatively low frequency, population screening studies are not cost-effective.

Key words: C282Y mutation, H63D mutation, HFE gene, hereditary hemochromatosis, transferrin saturation

INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder and the most common cause of iron overload. Iron overload is preventable, but can lead to significant long term sequale, inc-
including arthritis, hepatic cirrhosis, hepatocellular carcinoma, fatigue, heart failure, hypogonadism and diabetes mellitus if left untreated (1). The diagnosis of HH has improved since Feder et al. isolated the HFE gene in 1996 (The Human Leukocyte Antigen (HLA)-linked iron loading gene), which is located on the short arm of chromosome 6 on the HLA complex. (2). Two major HFE genes are the major allele C282Y (Cystein-282-Tryrosine or G845A) and the minor allele H63D (Histidine-63-Aspartate or C187G) (2,3), which present with variable frequencies in different populations. HH is the most common hereditary metabolic disorder in Caucasians and the overall prevalence of the condition among Caucasians of northern European extraction is about 5 per 1000 (4). Eighty to 90% of patients with HH have the C282Y variant, especially in homozygosity and 3% to 5% are compound heterozygotes between C282Y and H63D (5). The prevalence of C282Y homozygotes is at least 1 in 200 for people of northern European descent (6), and extremely rare in the African, Asian and Australasian populations.

Hereditary hemachromatosis is rarely diagnosed in Turkey and screening for HH in Turkish populations has been reported in only 4 prior studies, (7-10). The homozygote C282Y mutation has not been reported. The aim of this present study is to estimate the frequency of common HFE mutations in population of Central Anatolia.

MATERIALS AND METHODS

Study Design

A total of 2304 participants (1220 males, 1084 females) older than the age of 17 years old between December 2005 and December 2006 and applied to internal medicine departments. Written informed consent was obtained from all participants and the study protocol was approved by the Local Research Ethics Committee (Project number TT-06-15).

Exclusion Criteria

We excluded from the analyses those participants who had as following criteria; diagnosed and treated for prior HH, ingesting iron supplements, chronic hepatitis B or C patients, alcoholic liver cirrhosis, or patients who had thalassemi, sickle cell and sideroblastic anemia, porfuria cutanea tarda, malignancy or bone marrow insufficiency. The spot blood samples were collected and serum iron (SI) and totaly iron binding capacity (TIBC) were studied by the colorimetric method and transferin saturation (TS) for all samples were calculated (SI/TIBC*100=TS%). After an overnight fasting, blood samples were collected again from the participants whose TS was more than or equal 50% in man and more than or equal 45% in women. Serum ferritin levels and HH genetic analysis were also studied after an overnight fasting blood sample was obtained which met the above mentioned criteria.

In these participants, whole blood count and biochemical tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactic dehydrogenase (LDH), glucose, creatinine, bilirubin, cholesterol) were analyzed. These participants also underwent a complete physical examination and a detailed medical history was obtained for symptoms or signs of HH.

DNA Isolation and Mutation Detection

Six ml of peripheral blood samples were collected from each participant and DNA isolation was performed with Qiagen isolation Kit according to manufacturer’s instructions. The frequency distribution of C282Y and H63D in 14 participants was determined using PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Long Polymorphism) method. The PCR primers for amplifications of the Cys282Tyr locus used were: C282Y forward (5' TGG CAA GGG TAA ACA GAT CC 3') and C282Y reverse (5' CTC AGG CAC TCC TCT CAA CC3'). For detection of His63Asp, we used the H63D forward (5' ACA TGG TTA AGG CCT GTT GC 3') and H63D reverse (5' GCC ACA TCT GGC TTG AAA TT 3').

Statistical Analysis

SPSS 11.0 software was used to perform statistical analysis. In measured data, distribution was defined as a mean±standart error. Prevalences were calculated. And the relationship between variables were evaluated using the Pearson-Spearman correlation coefficient. A p-values less than 0.05 were considered statistically significant.

RESULTS

A total of 2304 participants were included, 1220 (53%) were male and 1084 (47%) were female. The mean age of men was 41.4±13.7 (range 17-78 years, median 41) and the mean age of woman was 42±14.3 (range 17-83 years, median 41). Mean SI of men was 62.87±32.6 μg/dL (3-211 μg/dL) and mean TS of men was %21.16±12.3 (1-96%). Mean
SI of women was 50.58±29.6 μg/dL (range 1-178 μg/dL) and mean TS of women was 15.15±11.01 (range 1-85%). No significant correlation between SI or TS and age was identified (r=-0.02, p=0.32 and r=-0.01, p=0.68, respectively). TS was more than or equal to 50% in 23 (1.88%) men and more than or equal 45% in 16 (1.48%) women. In this population, SI and SIBC were studied after an overnight fasting blood samples and TS was calculated. Control TS was more than or equal 50% in 9 (40%) men and more than or equal 45% in 5 (31%) women. Mean serum ferritin were 182.19±72.4 ng/mL (62–754 ng/mL) in men and 46.4±12.54 ng/mL (21.2–78 ng/mL) in women. In this group, Spearman correlation coefficient was calculated between age and ferritin due to numerical minority and a correlation between age and ferritin was detected (r=0.77, p=0.001).

HFE gene mutations were analyzed in 9 men and 5 women, C282Y homozygote mutation were found in 1 of 9, C282Y heterozygote mutation were found in 1 of 9 patients (Figure 1). No homozygote or heterozygote H63D mutation was found in men. H63D heterozygote mutation were found in 1 of 5 women (Figure 2) and the homozygote H63D, homozygote C282Y, and the heterozygote C282Y gene mutations was not detected in this population. There was no compound heterozygote (C282Y/H63D) mutation in either males or females (Table 1, 2). The homozygote C282Y mutation and heterozygote C282Y mutation prevalences calculated as

**Table 1.** Spot and fasting TS%, ferritin values and gene analysis of the HFE

<table>
<thead>
<tr>
<th>No</th>
<th>Patient</th>
<th>Age</th>
<th>SD (μg/dL)</th>
<th>TIBC (μg/dL)</th>
<th>TS %</th>
<th>Fasting TS %</th>
<th>Ferritin (ng/mL)</th>
<th>C282Y</th>
<th>H63D</th>
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<tr>
<td>1</td>
<td>Female</td>
<td>40</td>
<td>167</td>
<td>268</td>
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<td>195.7</td>
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<td>-/-</td>
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<td>-/-</td>
<td>-/-</td>
</tr>
<tr>
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<td>+/-</td>
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<tr>
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<td>66</td>
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<td>-/-</td>
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<td>320</td>
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<td>50</td>
<td>78</td>
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<td>239</td>
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<td>11</td>
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<td>302</td>
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<td>110.6</td>
<td>-/-</td>
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</tr>
</tbody>
</table>

+/+: homozygote-mutation (+). +/-: heterozygot-mutation carrier. +/-: normal (wild tip)-mutation (-).
0.08% (1/1220) in both male and female participants. The heterozygote H63D mutation prevalence was found as 0.09% (1/1084) in female participants. Calculated prevalences in the general population are as follows; the homozygote C282Y mutation prevalence is 0.043% (1/2304), the heterozygote C282Y mutation prevalence is 0.043% (1/2304) and the heterozygote H63D mutation prevalence is 0.043% (1/2304).

In the men who are C282Y homozygotes, and other C282Y heterozygote had ferritin levels of 754 ng/mL, and liver biopsy was suggested but they did not accept. All of these patients had no phenotypical properties of HH. In HH patients found to be C282Y homozygote, therapeutic phlebotomy was started and in one patient found to be a C282Y heterozygote (carrier) were educated about iron accumulation, and observed for a 3 year period.

**DISCUSSION**

HH is a autosomal recessive genetic disease of inappropriate intestinal iron absorption leading to iron overload and end-organ disease (11). The disease is most prevalent in white individuals of Northern European descent where the prevalence is 1 in 5000. The C282Y and H63D mutations on the HFE gene accounts for 90% of HH. The prevalence of C282Y homozygosity occurs approximately in 1 case per 200 persons (12-16). Clinical signs attributed to HH include liver failure, arthralgia, chronic fatigue, diabetes mellitus and congestive heart failure. Therapeutic phlebotomy to deplete excessive iron stores is the standard treatment of HFE-related HH, and organ failure can be prevented by phlebotomies starting before irreversible damage has occurred (17). Asberg et al. reported through assessment of general health status that there was no significant difference between normal healthy persons and homozygote C282Y persons who underwent maintenance phlebotomies (18). Screening to facilitate early diagnosis is desirable in individuals at risk of developing HH-related iron overload.

In countries with a high prevalence of C282Y gene mutation the first recommended test for screening of the HH (Hereditary-familial hemochromatosis) disease is genetic analysis, and then looking TS. In countries with low prevalence of the C282Y gene mutation it would be correct to use TS as a primary screening test (19). Due to its easy applicability, low cost, high sensitivity and acceptable specificity the two-step (spot and after fasting) TS measurement is still the most widely used and the most appropriate screening test for HH (20,21). In studies, a cut off value of 45% for TS had a sensitivity of 94–98% (14,22). DNA testing is used to show gene mutation is the test with the highest specificity and positive predictive value. In this study, after measuring fasting TS and serum ferritin levels, genetic evaluation based algorithm was applied to individuals suspected of HH. In one prior study by Cogswell et al., it has been reported that when the threshold value for TS is increased to 62% the sensitivity of the test is found to decrease whereas the specificity increased in men (23). When the sensitivity is lower, the probability of the individuals in the population likely to be missed is increased so a cut off value was used as 50% for men and 45% for women in this study. The control TS values of most participants in our study after an overnight fasting, was considered normal. Fasting was necessary to prevent artificially high, do to iron consumption before spot blood sample was taken. A study with 11 women and 10 men who did not have the HH disease although who had iron overload, it was reported that, the most common cause was excess dietary iron intake, excessive alcohol consumption, and other liver diseases (24). Screening studies for HH have been conducted on various groups such as workers (25,26), volunteer blood donors (27,28), patients followed in a hospital (29,30), and people who are living in a particular geographic region (14). In order to minimize
the patient contact, outpatient population who came to the hospital for any reason were screened. Our study reflects not only Kayseri population but also reflects approximately a population of three million including the neighboring provinces of Kayseri.

In this study it was observed that there was no relationship between the SF and TS values and age ($r = -0.01$, $p = 0.68$). Similarly in the previous studies it has been reported that there was no association between age-TS, and age-SF (8,24). Gonzales et al. detected that SF and TS levels were higher in men than women, and it was reported that incidence of excessive iron accumulation increases with age, and there was a positive correlation between advanced age, male gender and iron accumulation (31). Gonzalez and colleagues results supported earlier studies and they reported that serum ferritin levels in men had been identified higher than in women (31–33). A possible cause for this is because of pregnancy, lactation, due to blood loss with menses and the accumulation of excess iron was fewer in women with late-onset of excess iron accumulation was observed (34). In both sexes an increase in the level of ferritin with age was observed (15). Similar to previous studies also a correlation between age and serum ferritin levels was revealed ($r=0.77$, $p=0.001$).

In the general population, the C282Y homozygous mutation frequency whose relationship between HH phenotypic expression had been the most well-known, was detected as 0.043% (1/2304) in this study. In Northern Europe the majority of the patients with the diagnosis of HH, have this genotype (2, 29, 35-37). However in Central Antolia, screening studies conducted in Istanbul, Ankara and Izmir have reported that the homozygous C282Y gene mutation is absent (8-10, 38, 39). Only one familial case report series has been reported with homozygous C282Y gene mutation from Turkey (40).

Many factors play a role in the phenotypic expression of a genetic defect. The factors which are related to the patient such as age, gender, genetic modification are important while acquired factors such as diet, alcohol use, are effective in the emergence of the disease (1,41-43). Serum ferritin levels may not always found to be high in C282Y homozygous individuals. A decrease in the value of serum ferritin levels in acute illnesses, even in individuals homozygous for C282Y has been observed (29). Four recent studies of HH, it was detected that in C282Y homozygous individuals normal ferritin levels rates between all homozygotes were found as 25%, 40%, 50% and 80% respectively. However, in a recent study by Adams and colleagues of individuals who volunteered for blood analysis (28), ferritin and TS values could not reflect the seriousness of the disease completely. In the study which was conducted by Sanchez and his friends in Spain, 5370 healthy voluntary blood donor subjects, (3467 males, 1903 females) were evaluated for HH and they have found 8 C282Y homozygous patients (0.15%) and 74 compound heterozygous (C282Y/H63D) patients (1.38%). Eight patients who were C282Y homozygous (five males and three females) reported none of the symptoms at diagnosis (34). In our study we identified a male patient who had a homozygous C282Y mutation. Although his TS was high, ferritin level was within normal limits. In addition, liver function tests were normal. Symptoms and were negative for HH.

Liver biopsy is the gold standard for the diagnosis of HH. Bacon and colleagues, found in their study that there had been no fibrosis in liver biopsies of the patients whose liver enzymes were normal and who was under 40 years of age. Liver biopsy is not recommended for C282Y homozygous patients who are under 40 years of age and whose liver enzymes are normal and serum ferritin is below 1000 ng / mL (21, 45). In a study of 197 C282Y homozygous patients in France, Guyader and colleagues found that there were severe fibrosis in liver biopsy in patients with normal AST levels without hepatomegaly by physical examination and whose ferritin levels under 1000 ng/mL (44). Liver biopsy was recommended for patients with the C282Y homozygous genotype but the patient did not accept it.

In this study, C282Y heterozygous mutation were found in a men. It has been reported that no obvious phenotypic difference was observed between C282Y heterozygotes and wild-type (normal) individuals and there was no difference between their serum iron and ferritin concentrations (46,47). The C282Y heterozygous patient was asymptomatic, even though he did not show signs and symptoms of HH disease, he had very high serum ferritin values. These findings are opposed the previously mentioned findings, indicating that there is a relationship between iron overload and C282Y heterozygosity mutation. Another possible explanation the reason for this (infection, malignancy, hepatitis, etc..) was not detected and the patient
with the preliminary diagnosis of HH was suggested to go under liver biopsy but the patient did not accept it.

Heterozygous H63D mutation was detected in a female participant whereas homozygous H63D or compound heterozygous (C282Y/H63D) mutation was not found. In a study conducted in Spain, the reported H63D allele frequency was 20.8%±1.09 (homozygous for H63D is 1/23) and H63D carrier frequency was 32.9% (1/3), and these showed that H63D allele frequency and H63D carrier frequency present in significant percentage of the community. These frequencies were significantly difference between women and men (34). Although there was no relationship between H63D heterozygosity and iron accumulation, slight accumulation of iron is observed in H63D homozygous patients. Moirand et al. suggested that homozygous H63D mutation was not enough to cause accumulation of excess iron unless there were additional factors such as alcoholism, metabolic diseases, such as C282Y heterozygosity (48). We detected no signs of iron accumulation in the patient with heterozygous H63D mutation.

The incidence of homozygous C282Y mutation was 0.043% (1/2304), heterozygous C282Y mutation frequency was 0.043% (1/2304) and H63D heterozygous mutation was 0.043% (1/2304) in the general population. These results resembles the previous screening studies conducted in our country, which found a lower prevalence. The first study was conducted by Servet Ozaydin from Gulhane Military Medical Academy in 1994. In this study 1000 healthy men between the ages of 19-42 were screened for HH by looking TS and clinical findings but made no patient individual could be identified (7). Meryy-Clark and colleagues had observed the frequency of C282Y and H63D mutations in study group consisted of different ethnic groups, including a total of 70 Turks in 2000. In this study, C282Y mutation causing the disease was not detected and the frequency of H63D mutation was reported as 14% (6). The study conducted by Gültekin Barut in Istanbul in 2001, 4633 people (3827 males, 806 females) were evaluated in terms of HH. The heterozygous H63D mutation and homozygous H63D mutation frequencies were found as 0.03% (1/3827) of men and as 0.31% (12/3827) respectively. Heterozygous H63D mutation frequency was detected in 0.12% (1/806) of women while there was no woman identified with a homozygous H63D mutation. The prevalences of hetrozygous and homozygous H63D mutations in the general population were calculated as 0.28% (13/4633) and 0.021% (1/4633) respectively. No C282Y mutation was found in either sex (8). Taylan Demirci conducted a study in Izmir in 2002 with 730 participants. The group was composed of 126 patients with TS values of ≥45% while 604 patients with TS values of <45% of them formed the control group. C282Y mutant allele and H63D frequencies were found 1.16% and 24% respectively, in the patients with TS values >%45 while C282Y frequency of the mutant allele and H63D frequencies were found as 0% and 18% respectively, in the individuals with TS values <45 (10). In 2002, Bektas et al, in Ankara designed a screening study on the 3060 healthy people who had donated blood and who were over the age of 20. In this study, reference value of <28 mmol /L for unsatureted iron binding capacity (UIBC) was used as the initial test. The participants whose UIBC were identified as low were repeated fasting UIBC and were looked ferritin values and then genotype studies liver biopsies were done. It had been reported that, 75 individual had UIBC <28 mmol / L but none of them had high ferritin levels. Fasting UIBC repeated for 65 patients, and again for 5 patients (8%) whose UIBC levels were the lowest in the genotype studied. Heterozygous H63D gene was detected in two individuals while none of homozygous C282Y or H63D had been reported. Liver biopsy was recommended in four of the five patients. Three of them had steatosis and a patient who had HBsAg positivity had hepatic periportal inflammation. By atomic-absorpti on spectrophotometry it was identified, that none of these four patients had increased liver iron content. The next stage, for the individuals with normal fasting UIBC levels but whose baseline UIBC values were low are genotype studies. Using genotype studies seven patients (11.6%) had heterozygous H63D while none of the patients had homozygous C282Y or compound heterozygous present. Bekttaş and colleagues proposed that community screening studies for hemochromatosis in Turkey is not indicated, (9).

Not all patients with the diagnosis of homozygous HH will develop clinical manifestations of the disease in the future. For this reason, it is recommended that, all of the cases diagnosed by the screening study without phenotypic expression should not be taken to treatment protocol but should be closely followed-up (24). In a previous study when TS levels of homozygous C282Y were compa-
red with TS levels of compound heterozygous (C282Y/H63D) or wild-type, it was found that homozygous C282Y people had a wide range of TS levels increasing over time (49). Asymptomatic homozygous patients, followed up with preventive phlebotomy continue living normally with general population. Due to ethical reasons, it is not possible to follow-up asymptomatic homozygotes without applying a treatment protocol by long-term prospective randomised studies (21, 44). Today, HH-related mortality rate is low, but still, some patients are dying with hepatocellular cancer, cardiomyopathy, cirrhosis, or dying due to complications of diabetes. Therefore, in this study therapeutic phlebotomy sessions began to the asymptomatic individual who had C282Y homozygous mutation and after 3-year follow-up, the patient did not develop any complication related to HH.

In conclusion, this study has similar findings with earlier studies from our country. In our society, the most common gene mutation which is HFE gene mutation associated with HH is quite low compared with the HFE gene mutation in Northern European countries. It has been reported that although development of life-threatening illness are expected to occur in 20% of patients it is cost-effective to use HH screening studies (44). Using blood taken from patients during treatment as blood donation may further reduce cost. It was reported in the last meeting of specialists that it is possible to use blood or blood products of the patients with the diagnosis of HH (19). However, this approach and cost-effectiveness claim are valid for screening studies of the populations with a high incidence of HH. It is still controversial in Central Anatolia due to low prevalence of the disease. (50). Large population screening studies for HH, if it is associated with health surveillance programs, may be more cost-effective. For the moment, large community surveys do not seem to be possible, however improving awareness for HH among the public and healthcare community is imperative.

REFERENCES


