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Iranian hereditary hemochromatosis patients: Baseline characteristics, laboratory data and gene mutations

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Summary

Background:

Hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in white people, characterized by highly abnormal uptake of iron from the gastrointestinal tracts. Recently, mutation studies have focused to detect the genes responsible for HH.

Material/Methods:

In this cross-sectional study, 12 HH patients were recruited, who were referred to Firoozgar Hospital, Tehran, Iran. In addition to the clinical assessments, a complete laboratory evaluation, imaging modalities, histopathologic assessment, atomic absorption spectrophotometry and gene mutation study were performed. The genetic study for HFE gene mutation was examined for all of the patients since 2006, while non-HFE mutation was conducted since December 2010 (only for 1 of them).

Results:

Twelve patients were evaluated consisting of 11 men and 1 woman, with the mean age of 39.58±12.68 yr. The average of atomic iron loads was 13.25±4.83-fold higher than normal standards. Four patients had heterozygotic mutation of H63D (33.3%). There was no significant difference in either the iron load of liver (P=0.927) and heart (P=0.164) or serum concentration of ferritin (P=0.907) and TIBC (P=0.937) between the HFE-mutant and without HFE mutation HH cases.

Conclusions:

In contrast to other studies, C282Y mutation was not detected in any of our Iranian HH patients. Heterozygotic mutations of H63D (HFE) and TFR2 (non-HFE) genes were found to be more common in these patients. Similar to previous reports, these mutations were not found to be significantly associated with severity of presentation in HH patients.

key words:

hereditary hemochromatosis • gene mutation • iron overload

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BACKGROUND

Hereditary hemochromatosis (HH) is an autosomal recessive disease that is characterized with excessive abnormal uptake of iron from the gastrointestinal tracts. Hence, it leads to storage of extra iron in the other organs, including liver, heart, endocrine organs, pancreas, synovium, and elsewhere. As a result, it can present with liver dysfunction, hypogonadism, arthritis, cardiomyopathy, and hyperpigmentation that can convert to severe disorders like hepato-cellular carcinoma (HCC), cirrhosis and heart failure [1–3]. In 1996, the first gene responsible for HH was discovered and called HFE. Feder et al reported that HFE exists on the short arm of chromosome 6 and produces protein that regulates transferrin receptor binding to the dietary iron [4]. Due to mutation of HFE, approximately 3 to 4 mg iron per day is absorbed through the intestinal tract instead of the normal 1 to 2 mg [2]. Thus, the accumulation of iron in various tissues occurs due to mutation of HFE [4]. Based on genetic mutations, HH has 4 types, including type 1 which is defined as HFE mutations including C282Y, H63D, S65C and the other types such as non-HFE mutations consisting of type 2a (mutation in hemojuvelin gene), type 2b (mutation in hepcidin gene), type 3 (mutation of type 2 transferrin receptor gene), and type 4 (mutation in ferroportin gene) [5].

HH is the most common autosomal recessive disorder among white people in Northern Europe, United States, and Australia [6,7], with an estimated prevalence of 1 in every 125,333 persons [8,9]. Type 1 is the most common type of HH [10] and can be seen in about 90% of patients [5]. The C282Y mutation is found in most Northern European populations [10–12], and most of the patients are homozygote [2,4,12]. Few studies have evaluated HFE mutation in the Iranian population, especially in HH patients, which are performed in different ethnic groups. The low prevalence of C282Y mutation was also reported in another survey in a sample of Iranian blood donors [13]. Although their gene mutation study was performed in healthy blood donors, C282Y and H63D mutations were evaluated in 1029 individuals, and showed the prevalence rates of 0.097% and 11.3%, respectively [13]. Another case report study on Iranian HH patients was performed by Nobakht et al. [14], in which H63D and C282Y mutations were not detected in 1 HH patient. Similar to these Iranian reports, in a recent case presentation of HH from China, HFE gene test showed a negative result for C282Y but was positive for H63D [15]. Recently, Milić et al. reported a mutation study in Croatian patients suspected of HH, and concluded that HFE mutations could be responsible for only 20% of HH in these patients [16].

Some studies reported that C282Y homozygote mutation has the major role in creating iron overload [16,17], whereas H63D homozygote mutation plays a lesser role. S65D mutation is a rare condition, frequently is presented compound with C282Y, which creates minor forms of HH [16]. On the contrary, non-HFE mutations seem to be more common in some populations in southern Europe [18,19] and Asia [20], where C282Y mutation is less likely to occur. An especially low frequency of C282Y mutation was reported from Iran [11,13,14].

Overall, in comparison to secondary hemochromatosis, which is due to several causes, HH is a rare disease in Iran

[14]. Few Iranian studies have focused on hereditary hemochromatosis and the prevalence of HFE gene mutation, which is mostly accompanied by other diseases like hepatitis, thalassemia and diabetes [11,13,21–23]. However, only 2 case reports have been published on HFE and non-HFE mutation in Iranian HH patients [14,24]. Due to the low prevalence of HH in Iran and low frequency of C282Y mutation as the most gene mutation of HH in the west countries, this study focused on 12 Iranian HH patients to evaluate their baseline characteristics, laboratory data, and gene mutations.

MATERIAL AND METHODS

Patients

In this cross-sectional study, 12 iron overload hemochromatosis patients were chosen: 11 men and 1 premenopausal woman, referred to Firoozgar Hospital, Tehran, Iran between 2006 and 2010. We asked gastroenterologists to refer patients who were suspicious of hereditary hemochromatosis from other health centers and clinics, consequently some of the recruited patients were from northern Iran. Moreover, Firoozgar Hospital, which is situated in the central part of Tehran, is to some extent considered as a major referral gastroenterology clinic.

The patients had to meet 2 main inclusion criteria: transferrin saturation (TS) of more than 45%, and serum ferritin of more than 400 µg/L in males and more than 200 µg/L in premenopausal women [25]. Exclusion criteria were: dyserythropoietic anemia, transfusions, porphyria cutanea tarda, thalassanemia major, sideroblastic anaemia, chronic hemolytic anemia, chronic hepatitis C disease, alcoholic liver disease, and medicinal iron use [17,26]. This study was approved by the Ethics Committee of Firoozgar Clinical Research Development Center (FCRDC), affiliated with Tehran University of Medical Science (TUMS). In addition, informed consent was verbally obtained from all patients, explaining the procedures and goals of this study.

Clinical evaluation was performed, including presence of hyperpigmentation (self-reported), arthritis (rheumatologist), arthralgia (self-reported), dyspnea (New York Heart Association (NYHA) Functional Classification) [27], impotency (endocrinology consult), and infertility (failure to conceive for one year without usage of any contraception methods). Complete laboratory evaluation included imaging modalities, histopathologic assessment, atomic absorption spectrophotometry and gene mutation study.

Laboratory methods

Biochemistry, Complete Blood Cell Count (CBC), Lipid Profile, Test (TFT), sexual hormones, serum iron, serum ferritin, Total Iron Binding Capacity (TIBC), and Liver Function Test (LFT) were measured in all the recruited HH patients. For this purpose, a 10 ml blood sample was obtained from each patient. Serum ferritin concentration was assayed using ELISA method with the Ideal Tashkhis Kit, Iran (ELISA reader, USA) [Normal range: 11–115 ng/ml in premenopausal women, 17–290 ng/ml in men]. Serum iron concentration was measured via photometry with the Biosystem Kit, Spain (autoanalyzer-Hitachi, Japan) [Normal range: 65–175

$\mu\text{g/dL}$ in men, 50–170 $\mu\text{g/dL}$ in women]. TIBC was determined by means of turbidimetry with a Biosystem Kit (turbidimeter-Hitachi, Japan) [Normal range: 250–410 $\mu\text{g/dl}$]. Afterward, TS was calculated by dividing serum iron concentration into TIBC [Normal range: up to 45%].

CBC analysis was performed, as well as the following indexes: White Blood Cell (WBC) [Normal range: 4000–10000/ μl], Red Blood Cell (RBC) [Normal range: 4.5–6.3 $10^6/\mu\text{l}$ in men, 4.2–5.4 $10^6/\mu\text{l}$ in women], Hemoglobin [Normal range: 14–18 mg/dL in men, 12–16 mg/dL in women], Hematocrit (Hct) [Normal range: 39–52% in men, 36–46% in women], Mean Corpuscular Volume (MCV) [Normal range: 77–97 fL], Mean Corpuscular hemoglobin (MCH) [Normal range: 26–32 pg], Mean Corpuscular Hemoglobin Concentration (MCHC) [Normal range: 32–36%] and Platelet count [Normal range: 140–440 $\times 1000/\mu\text{l}$].

LFT tests consist of Alanine Aminotransferase (ALT) [Normal range: 5–40 IU/L], Aspartate Transaminase (AST) [Normal range: 5–40 IU/L], Alkaline Phosphatase (Alk-P) [Normal range: up to 270 IU/L], Prothrombin time (PT) [Normal value: 12 sec], Albumin (Alb) [Normal range: 3.5–5.5 g/dL], total bilirubin [Normal range: 0.1–1.2 mg/dl] and direct bilirubin [Normal range: 0–0.4 mg/dl].

Other biochemistry tests were also considered, including Fasting Blood Sugar (FBS) [Normal range: 70–115 mg/dL], serum level of Low Density Lipoprotein (LDL) [Normal range: 60–130 mg/dL], High Density Lipoprotein (HDL) [Normal range: 30–75 mg/dL], Triglyceride (TG) [Normal range: 60–160 mg/dL] and Cholesterol (chol) [Normal range: up to 200 mg/dL]. Hormonal assessment was performed for each patient, including serum level of testosterone [Normal range: 0.3–12 ng/mL], Follicle-Stimulating Hormone (FSH) [Normal range: 2.9–12 mIU/mL in follicular phase in women, 1.2–13.2 mIU/mL in men], Luteinizing Hormone (LH) [Normal range: 0.7–10.8 mIU/mL in follicular phase in women, 0.8–7.8 mIU/mL in men], Thyroid-Stimulating Hormone (TSH) [Normal range: 0.4–6 $\mu\text{IU/mL}$], T_4 [4–12 $\mu\text{g/dL}$], and T_3 [1.3–3.1 nmol/L].

Imaging studies

Echocardiography and sonography were used to evaluate cardiac function and echogenicity of liver and spleen, respectively, for all patients. FibroScan was done for 2 patients who did not have indication for biopsy; this was performed in the Tehran Gastroenterology & Hepatology Center (TGHC), Tehran, Iran. Transient Elastography (TE) was done using FibroScan (Echosens, Paris, France). The procedures were performed on the right lobe of the liver. During the procedure, patients were lying in dorsal decubitus position with the right arm in maximal abduction. The operator, using an ultrasound guide, located a liver portion with a thickness of at least 6 cm and free of large vessels. The success rate was calculated as the ratio of the number of validated measurements over total number of measurements. The median value of at least 10 validated measurements was considered representative of the elasticity of the liver. The results were expressed in kilopascals (kPa). TE results with at least 10 validated measurements and a success rate of at least 60% were considered reliable. The fibrosis stage is based on the Metavir histological index [28], which classifies fibrosis on

a scale F0 to F4, where F0 is a normal liver without fibrosis and F4 represents liver cirrhosis.

Atomic absorption spectrophotometry to evaluate iron deposition in the liver was conducted on only 4 patients. However, since the atomic absorption spectrophotometry is considered a sophisticated operation, the cardiac and hepatic T2* Magnetic Resonance Imaging (MRI) was performed for 9 patients to measure iron load in these organs (2 of them were not available and another did not cooperate).

T2*MRI was done for 9 patients in the Noor Medical & Imaging Center in Tehran. MRI examinations were performed using a 1.5 Tesla Magnetom Siemens Symphony scanner (Siemens Medical Solution, Erlangen, Germany). Each scan lasted about 10–15 min. Standard circumstances were considered for both accuracy and patient safety. The MRI T2* of the liver was determined using a single 10 mm slice through the center of the liver, scanned at 12 different echo times (TE 1.3–23 ms). Each image was acquired during an 11–13 second breath-hold using a gradient-echo sequence with a repetition time of 200 milliseconds (ms), flip angle of 20°, base resolution matrix of 128 pixels, a field of view of 39.7 \times 19.7 cm, and a sampling bandwidth of 125 kHz per pixel. Estimation of iron loads was done using “CMR Tools” software. T2* (dry weight) value of >6.3 ms (<2 mg/g), 2.8 to 6.3 ms (2 to 5 mg/g), 1.4 to 2.7 ms (5 to 10 mg/g), and <1.4 ms (>10 mg/g) were considered as normal, mild, moderate, and severe hepatic iron load, respectively.

Electrocardiogram (ECG) gated cardiac MR images were also obtained for T2* calculation. Short-axis images were prepared in different sequences. Myocardial iron load with values of >20 ms, 14 to 20 ms, 10 to 14 ms, and <10 ms were defined as normal, mild, moderate and severe cardiac iron load, respectively.

Biopsy method

Liver biopsy was considered in those whose ferritin level was higher than 1000 $\mu\text{g/L}$, those who had elevated transaminase [29], and in the absence of any severe coagulopathy problems. Special staining was utilized to determine the iron deposition degree in hepatocytes as well as in the liver structure. Three of the patients did not have indications of biopsy; therefore, FibroScan was examined for 2 of them (1 patient was not available). As previously mentioned, FibroScan could be a replacement of biopsy to verify the liver construction.

The liver biopsy was done with 16-gauge needle (TSK Laboratory, Japan) for 9 patients who had the specified indications. The needle biopsies, which were at least 2 cm in length, were placed in Bowen solution. Hematoxylin & eosin staining by Perls' Prussian blue method was done to determine the grade of iron deposition in the hepatocytes. Iron deposition is graded 0–4 by Scheuer et al. [30]. Trichrome staining was also done to show connective tissue disarrangement. Hepatic Activity Index (HAI) score from 0 to 6 was used to determine the stage of these changes [31]. These procedures were performed at the Pathology Department of Firoozgar Hospital.

Gene mutation test

The genetic study results for HFE gene mutation was examined for all of the patients since 2006, while non-HFE

mutation was conducted since December 2010 (only for 1 of them). Since there was no sequencing of non-HFE genes technique in this center until November 2010, non-HFE mutation was assessed only for the last patient who was recruited after November 2010. Unfortunately, because of the expense of genetic study and also due to the long distances patients had to travel, it was not possible to recall them and recheck other patients for non-HFE mutations. Ten mL of blood was collected in EDTA from each patient and samples were sent to Kariminejad – Najmabadi Pathology & Genetics Center, Tehran, Iran, for genetic analysis. Genomic DNA was extracted according to established protocols [32]. Comprehensive analyses were carried out using reverse hybridization test strips (Vienna Lab Diagnostics, Vienna, Austria) to detect the following 12 mutations in the *HFE* gene: V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168X, W169X, C282Y, Q283P and E168Q. For 1 premenopausal patient who was referred after November 2010, non-HFE mutations were also checked, including additional 4 mutations in the transferrin receptor 2 (*TFR2*) genes (E60X, M172K, Y250X, and AVAQ594-597 del), and 2 mutations (N144H and V162 del) in the ferroportin 1 (*FPN1*) gene.

Statistical analyses

Data were analyzed using SPSS v.16 software (Chicago, IL, USA). For description of qualitative and quantitative variables, frequency percentage and mean \pm standard deviation (SD) were reported, respectively. One-sample Chi-square statistics was used to compare the prevalence of H63D in HH patients with that of healthy blood donors (as a historical control group) derived from a previous study [13]. Independent Sample *T*-test and Mann Whitney *U*-test were used to compare the mean of parametric and non-parametric continuous variables. Chi-square and Fisher's exact test were used to compare proportion of nominal or ordinal variables between groups of patients with or without HFE mutations, where applicable. In all analytical procedures, a *P*-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Finally, 12 patients were recruited into our study, consisting of 11 men and 1 woman, with the mean age of 39.58 ± 12.68 yr. The mean duration of their disease was 5.9 ± 3.14 yr. Table 1 illustrates the symptoms and signs observed during the study. The most common symptom was changing in skin color (75%), of which 50% had the local hyper pigmentation, and 25% had generalized hyperpigmentation. Moreover, impotency and infertility were seen in 41.7% (5 of 12) and 30% (3 of 10 married cases) of the patients, respectively.

Laboratory findings

Laboratory findings of the patients are summarized in Table 2. The mean level of serum iron was 250 ± 71.08 $\mu\text{g/dL}$, and the mean concentration of serum ferritin was 2642.75 ± 1275.39 ng/ml. The mean levels of ALT and AST were 69.75 ± 49.95 IU/L and 64.17 ± 46.69 IU/L, respectively. The results of atomic absorption spectrophotometry in 4 cases showed that the average of atomic iron loads

Table 1. Clinical characteristics, sign and symptoms of a sample of Iranian hereditary hemochromatosis patients (n=12).

Symptoms and signs	Number of cases	Percentage (%)
Skin hyperpigmentation:		
Negative	3	25.00
Local	6	50.00
General	3	25.00
Dyspnea:		
No dyspnea	4	33.30
Type 1	0	0.00
Type 2	5	41.70
Type 3	0	0.00
Type 4	3	25.00
Impotency	5	41.70
Infertility	3	30.00
Arthritis	8	66.70
Family history	6	50.00

was 13.25 ± 4.83 -fold higher than normal standards. Other laboratory findings are reported in Table 2. Hormonal assessment of the patients revealed that high TSH, low T_4 and low T_3 was presented in 1 (8.3%), 1 (8.3%) and 2 (16.7%) cases, respectively. Among male patients, low level of testosterone, FSH and LH each was observed in 27% of the patients (3 of 11). Only 1 male suffered from all of these 3 hormonal deficiencies.

Imaging studies

The findings of all imaging studies are presented in Table 3. In addition to these data, quantitative T2*MRI was performed to show iron overload in dry weight of liver; the mean loaded iron was found to be 3.66 ± 3.95 mg/g/dw, whereas ranges lower than 2 mg/g/dw are usually supposed to be normal.

Abnormal echocardiography and ultrasonography was seen in 5 (41.7%) and 9 (75%) patients, respectively. The detailed results of each modality are shown in Table 3. However, the mean of ejection fraction was $51.58 \pm 15.51\%$ and the most common ultrasonographic finding was changes in liver echogenicity (44.4%).

Fibro-scan study was also performed in 2 patients, showing the elasticity of the liver as 5 kPa and 7 kPa, both of which were in the normal range (F_0 - F_1).

Histopathologic study

Biopsy specimens were taken from 9 patients and evaluated according to HAI score and grading. The results showed fibrosis of stage III in 1 (11.1%), stage IV in 1 (11.1%), stage V in 2 (22.2%), and stage VI in 4 (44.4%) cases (ie, only 1 patient did not have any fibrosis). Regarding the grade of iron deposition in liver, 1 patient (11.1%) was in grade I, 3 (33.3%) in grade III, and the other 5 (55.6%) in grade IV.

Table 2. Laboratory findings of evaluated Iranian hereditary hemochromatosis patients (n=12).

Index	Minimum	Maximum	Mean	SD.
Serum ferritin (ng/ml)	785.00	5240.00	2642.75	1257.39
Serum iron (µg/dl)	150.00	369.00	250.00	71.08
TIBC (µg/dl)	252.00	450.00	315.33	68.26
Transferrin saturation (%)	49.00	110.00	79.07	18.87
FBS (mg/dl)	68.00	523.00	139.08	124.39
Hemoglobin (mg/dl)	10.40	19.60	14.13	2.52
Hematocrit (%)	34.80	49.10	41.58	4.12
MCV (fl)	65.00	100.70	82.85	12.49
MCH (pg)	18.00	33.10	26.82	5.43
MCHC (%)	28.90	34.40	31.89	1.99
RBC count ($\times 10^6/\mu\text{l}$)	3.20	6.70	4.89	1.07
WBC count (/µl)	3700.00	8700.00	6275.00	1416.22
Platelet count (/µl)	69000.00	362000.00	205666.00	78043.50
INR	1.00	1.60	1.15	.20
PT (sec)	11.50	19.00	13.41	1.98
ALT (IU/L)	23.00	197.00	69.75	49.95
AST (IU/L)	17.00	146.00	64.17	46.69
Alk-P (IU/L)	143.00	879.00	307.83	209.91
Total Bilirubin (mg/dl)	.30	6.40	1.68	1.68
Direct Bilirubin (mg/dl)	.10	4.60	.82	1.31
Alb (g/dl)	2.30	6.80	4.23	1.09
LDL (mg/dl)	66.00	163.00	109.42	30.44
HDL (mg/dl)	23.00	65.00	43.92	13.80
Triglyceride (mg/dl)	68.00	367.00	169.67	87.80
total cholesterol (mg/dl)	129.00	304.00	194.58	51.72
Atomic iron load (-fold)	9.50	20.00	13.25	4.83

Genetic study

Genotype evaluation was done to detect mutation of HFE and non-HFE gene since 2006 (in all 12 cases) and 2010 (only in 1 patient), respectively. Four patients had heterozygotic mutation of H63D (33.3%). Only 1 patient, who non-HFE was assessed in, was homozygote mutant for AVAQ594-597 del of TFR2 gene. Compared with previously established prevalence of H63D mutation in Iranian healthy blood donors (as a historical control group) [13], the observed H63D mutation was significantly higher in our HH patients (33.3% vs. 11.3%, $P=0.013$).

In Table 4, iron-related indexes and clinical data are compared between patients with or without HFE mutation, showing only non-significant differences between these 2 groups

(all P -value >0.05), except for infertility, which was significantly higher in patients with any HFE mutation (75% vs. none, $P=0.048$).

DISCUSSION

Genetic study was performed for all of the patients. Twelve mutations of HFE gene (since 2006), 4 mutations in TFR2 gene, and 2 mutations in FPN1 gene of non-HFE gene (since 2010) were considered. Five patients have mutation disorders. One female had homozygote mutation of AVAQ594-597 deletion in TFR2 of non-HFE gene, and the rest of the mutant patients were heterozygote in H63D mutation of HFE gene. Previous research revealed that the presentation of TFR2-HH is similar to HFE-HH, but makes iron overload within another gene dysfunction. Although this is

Table 3. Findings of different imaging studies in a sample of Iranian hereditary hemochromatosis patients (n=12).

Modality	Number of cases	Percentage (%)
Liver MRI		
Normal	5	55.60
Iron overloaded	4	44.40
Mild	1	11.10
Moderate	2	22.20
Severe	1	11.10
Heart MRI		
Normal	6	66.70
Iron overloaded	3	33.30
Mild	1	11.10
Moderate	1	11.10
Severe	1	11.10
Echocardiography		
Normal	7	58.30
Abnormal	5	41.70
LV systolic dysfunction	1	20.00
LV diastolic dysfunction	1	20.00
Both of them	3	60.00
Ultrasonography		
Normal	3	25.00
Abnormal	9	75.00
Enlargement of liver	1	11.10
Enlargement of spleen	1	11.10
Changing echogenicity of liver	3	33.30
Enlargement of liver & changing echogenicity of spleen	2	22.20
Enlargement of splee & changing echogenicity of liver and spleen	1	11.10
Enlargement of spleen & changing echogenicity of spleen	1	11.10

Table 4. Comparison of iron-related indexes and clinical data between HH patients with or without HFE mutations.

Index	Without HFE mutation (n=7)	With HFE mutation (n=4)	P-value
MRI of the liver (ms)	8.73±10.90	9.41±7.09	0.927
MRI of the heart (ms)	28.46±12.76	15.86±5.42	0.164
Serum ferritin (ng/ml)	2580.57±1538.46	2480±587.20	0.907
Serum iron (µg/dl)	249.00±87.92	256.75±53.59	0.878
TIBC (µg/dl)	322.43±62.62	318.75±88.16	0.937
TS (%)	75.41±19.34	82.50±21.52	0.588
Skin hyperpigmentation (%)	28.6%	25.0%	
Negative	42.8%	75.0%	
Local	28.6%	0.0%	0.441
General	28.6%	50.0%	
Dyspnea (%)	0.0%	0.0%	
No dyspnea	42.8%	25.0%	
Type 1	0.0%	0.0%	
Type 2	28.6%	25.0%	0.757
Type 3	28.6%	75.0%	
Type 4	0.0%	75.0%	
Impotence (%)	57.1%	75.0%	0.242
Infertility (%)	57.1%	50.0%	0.048*
Arthritis (%)			1.000
Family History (%)			1.000

* P<0.05 indicates significant statistical difference.

a low-frequency mutation, it affects younger patients and may produce more severe clinical features [33]. By contrast, it was shown in our research that TFR2-HH may produce mild clinical phenotype in a 44-year-old premenopausal woman, perhaps due to blood loss during her periodic menstrual cycle.

None of these Iranian patients had C282Y mutation, which is known as the most common genetic cause of hereditary hemochromatosis in Western countries [10–12]. More recently, Yavarian et al. [11] have performed another genetic study on 170 southern Iranian persons, including 9 HH patients. They detected H63D and C282Y homozygous mutations in 4 and 1 HH cases respectively, while other 2 had compound forms of H63D and C282Y mutations, and 1 patient had C65D and H63D mutations. Also there was 1 heterozygous H63D mutation among the HH patients. Their HH patients were from the southern part of Iran, whereas our cases were mostly from the north; and this difference may account for the varied findings of these 2 studies. Recently, another report from southern Iran by Jowkar et al. [34] showed no cases of either a homozygous or heterozygous C282Y mutation; however, heterozygosity of H63D mutation was detected in 22% of patients with cryptogenic cirrhosis and in 28% of the normal population. Either our study or Yavarian's [11] or Jowkar's [34] are not representative of different Iranian ethnic groups. As mentioned, their samples mostly came from the south of the country, while ours were generally referred from the northern parts of Iran.

One of the recruited patients in our study died 4 years ago — he was a diabetic 50-year-old man with cirrhosis and cardiomyopathy (EF=30%). He was heterozygote in H63D mutation. When he was studied, non-HFE mutations were not considered. It is anticipated that there would be additional causes for this death beyond being heterozygote in H63D mutation. Although H63D is more frequent than C282Y mutation [35], some research in this area suggests the H63D mutation has fewer effects on iron overload disorders [2]. Even homozygous H63D mutation is reported to have no significant role in abnormal metabolism of iron [36]; however, in the previous study it was illustrated that serum ferritin level does not change along with HFE mutations [37]. Clark et al. [17] reported that only the homozygote mutation of C282Y and heterozygote compound mutation can increase probability of iron overload within the other HFE gene mutations. As a whole the following order estimates the effect of HFE gene mutations on severity of iron overload: 1) C282Y/C282Y, 2) C282/H63D, 3) H63D/H63D, 4) heterozygotic form of C282Y, and 5) heterozygotic form of [38]. Iron overload might be present even in non-HH individuals with higher prevalence of HH genetic predisposition, genotypes including C282Y homozygotes, and compound heterozygotes (C282Y/H63D) [39]. However, the comparison of iron-related indexes in our study (including serum ferritin, TIBC, and iron overload in heart and liver) showed no significant difference, mostly due to small sample size. However, the adverse event of infertility was significantly more likely to be observed in the HFE mutant group, even though the sample size was very low. By contrast, a recent population-based study in China compared HFE mutations in 444 infertile men versus 423 controls with proven fertility, showing no association between H63D mutation and idiopathic male reproductive dysfunction [40].

Since H63D mutation is considered as a usual variation in normal white people [41], it is suggested from previous findings and the present study that all the mutations should be taken into account. Results of the present study suggest there is a need to sequence some new HFE and non-HFE genes to find out more mutations that may cause this disease in light of recent evidence, not just the 12 mutations of HFE and the 6 mutations of non-HFE II genes. Future research should investigate environmental factors and other genes mutations that may play a significant role here. Some of the environmental risk factors that help to express the mutative genes to make HH include: HCV or HBV disease, high alcohol consumption, a diet high in red meat, drug induced, consumption of mineral supplements and vitamins (especially vitamin C) and eating food covered by mycotoxins [44]. Almost all of these risk factors are preventable to avoid probable disease. As genetics has an important role in HH, and there were familial links between some of our patients, it is useful to evaluate their first-degree family to detect serum iron and serum ferritin level. While no mutation was found in our 7 patients, it seems that genetic study limited to HFE gene mutations is not an economical way to screen Iranian people for HH. Genetic study is indicated if an iron overload patient has clinical features or for every person who has family history of HH accompanied gene mutation [17].

Due to the high prevalence of mutation of beta thalassemia in Iran, especially near the Caspian Sea and Persian Gulf (about 10%) [45], 4 of the northern patients suspected to have minor beta thalassemia. Three of them have had MCV lower than 70 fL, but normal hemoglobin electrophoresis of minor beta thalassemia; while only 1 patient had MCV<70 fL and abnormal hemoglobin electrophoresis of minor beta thalassemia. We could not be sure that minor beta thalassemia might be the reason of this heavy iron overload because ferritin could not arise up to 2000–3000 ng/ml in minor beta thalassemia and absence of minor beta thalassemia as a cause of HH in the literature. Therefore, considering the exclusion criteria, we included them as HH patients. Of course, evaluation of genetic study should be reconsidered to find probable non-HFE mutations or other HFE mutations. Other limitations of our study include small sample size (due to the low prevalence of HH in Iran) and not being representative of the whole country's HH population (as 8 of our cases were referred from the north of Iran). Moreover, sequencing of non-HFE gene was done only for 1 HH case, and our results were compared with historical control data from healthy Iranian blood donors; it was neither possible nor cost-effective to directly reassess the prevalence of these mutations in healthy controls.

CONCLUSIONS

Diagnosis of HH should be based on clinical features, laboratory findings, imaging studies, biopsy results and get heal by phlebotomy (as all of our patients become better at phlebotomy and using deferoxamine). Sequencing of the non-HFE gene mutations can help to confirm the disease. Our results illustrate the complications and symptoms of iron overload. They were similar to the populations from the west [1–3], but the gene mutation pattern differed [10–12]. Although non-HFE mutations and other HFE mutations were not checked in our samples, a different pattern

of gene mutations compared with Western countries could be seen as the most important finding of our study (referring to those 12 HFE-evaluated mutations in our study). It seems that HFE mutation sequencing alone is not a sufficiently useful way to screen HH in the Iranian population, especially in the northern part of the country.

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