



Research Article

Should HFE mutations be checked in polycythemic patients even at lower iron levels?

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Abstract

Objectives: The relationship between polycythemia and hereditary hemochromatosis (HH) has been investigated in several studies. This study aimed to evaluate the association between iron parameters and Hemochromatosis Protein (HFE) gene mutations in patients with primary or secondary polycythemia, as well as in non-polycythemic patients with elevated iron parameters.

Methods: A total of 106 patients who were evaluated for polycythemia or underwent HFE mutation testing due to elevated transferrin saturation (TS) and ferritin levels in the hematology department between 2015 and 2022 were retrospectively reviewed.

Results: The median age of the 106 patients (77 male, 29 female) was 54 years (range, 19–83). HFE gene mutations were detected in 44 patients (41.5%; 31 male, 13 female). Thirty-seven patients (35%) with Myeloproliferative Neoplasms (MPNs) were classified as Group 1, 52 (49%) with secondary polycythemia as Group 2, and 17 (16%) who underwent HFE mutation testing due to elevated TS/ferritin levels without polycythemia as Group 3. The mean TS level in Group 1 was significantly higher than in Group 2 ($p=0.032$). Among HFE(+) patients, mean TS was significantly higher in Group 3 compared with Group 2 ($p=0.023$). When all polycythemic HFE(+) patients (primary + secondary) were compared with non-polycythemic HFE(+) patients, mean TS was significantly higher in non-polycythemic patients ($p=0.026$).

Conclusion: The relatively high frequency of HFE positivity in patients with secondary polycythemia, together with its association with lower TS levels, suggests that the possibility of HH should not be overlooked in secondary polycythemia, even at lower TS levels.

Keywords: Hemachromatosis, hemochromatosis protein (HFE), polycythemia, , transferrin

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Hereditary hemochromatosis (HH) is an autosomal recessive disease that disrupts iron metabolism and leads to iron accumulation in the parenchymal cells of tissues. It mainly affects the liver, heart, and pancreas. The HFE gene is located on the short arm of chromosome 6 (6p21.3). Typical HH

patients carry two copies of the C282Y mutation in the HFE gene. The C282Y homozygous mutation is the most common genotype seen in HH, with a frequency of 80–85%. A minor HFE mutation is found in the H63D genotype, either in the homozygous form or as a compound heterozygous (C282Y/

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H63D) variant. Most patients present with normal serum iron tests and only mild to moderate iron accumulation [1].

Ferritin is the most commonly used biomarker of systemic iron stores in clinical practice. If serum ferritin exceeds 300 ng/mL, systemic iron overload may be considered. However, ferritin expression can be influenced not only by serum iron, but also by inflammatory cytokines, hormones, and oxidative stress [2]. Therefore, professional societies recommend screening for transferrin saturation ($TS = \text{serum iron} / \text{TIBC} \times 100$) in both asymptomatic and symptomatic patients with suspected hemochromatosis. If the TS is >45%, with or without hyperferritinemia, further testing for HFE gene mutations is recommended [3].

The World Health Organization (WHO) defines polycythemia as hemoglobin (Hgb) >16.5 g/dL and/or hematocrit (Hct) >49% in men, and Hgb >16 g/dL and/or Hct >48% in women [4]. Polycythemia vera (PV) is a Myeloproliferative Neoplasms (MPNs) characterized by clonal proliferation of myeloid cells, with 95% of patients harboring the JAK2 V617F mutation. Although no direct association has been established between PV and HH, the coexistence of the two disorders has been reported in a small number of case studies [4, 5].

Secondary polycythemia may occur due to elevated erythropoietin levels in conditions such as chronic obstructive pulmonary disease (COPD), cyanotic right-to-left cardiac shunts, sleep apnea, high altitude, chronic carbon monoxide intoxication, post-renal transplantation, polycystic kidney disease, hepatocellular carcinoma, renal cancer, and certain brain tumors [6].

Even when no cause for secondary polycythemia is identified, it has been suggested that the presence of erythrocytosis in HH patients with polycythemia may be secondary to increased iron uptake by erythroid precursors in the bone marrow, which may or may not be transferrin-dependent [7].

In this retrospective study, in contrast to previous reports, we aimed to investigate the relationship between transferrin saturation and serum ferritin levels in patients with primary or secondary polycythemia undergoing HFE mutation testing, as well as to evaluate iron kinetics and the presence of HFE gene mutations in patients without polycythemia.

Materials and Methods

The study was approved by the Local Ethics Committee for Clinical Research of Izmir Katip Çelebi University Atatürk Training and Research Hospital (Committee approval dated 21.03.2023 and decision number 0054). The research was conducted in accordance with the "WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects".

We retrospectively reviewed the iron kinetic parameters and mutation results of patients who underwent HFE mutation testing between January 2015 and January 2022 at the Department of Hematology, Izmir Kâtip Çelebi University Faculty of Medicine Hospital.

For all patients, complete blood count (hemoglobin, hematocrit, white blood cell, neutrophil, and platelet counts) was per-

formed using an automated hematology analyzer (XN-1000, Sysmex Corporation, Kobe, Japan). Serum ferritin levels were determined by chemiluminescence immunoassay (Dxl 800, Beckman Coulter Inc., USA). Erythropoietin (EPO) levels were measured by chemiluminescence immunometric analysis on the Immulite 2000 system (Siemens Healthineers, Germany). Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, serum iron, and total iron-binding capacity (TIBC) were analyzed using a fully automated biochemistry analyzer (AU5800, Beckman Coulter Inc., USA).

Genetic testing included analysis of HFE C282Y and H63D polymorphisms as well as JAK2 exon 14 and exon 12 mutations. Genomic DNA was extracted from peripheral blood leukocytes using a column-based purification kit (Qiagen, Hilden, Germany). Genotyping for HFE mutations was performed by real-time polymerase chain reaction (PCR) with allele-specific probes using commercial kits (Genviset HFE H63D and Genviset HFE C282Y, Qiagen). JAK2 mutation analysis was carried out by real-time PCR using the Ipsogen JAK2 MutaQuant Kit (Qiagen) according to the manufacturer's instructions.

Demographics, comorbidities, smoking history, presence of splenomegaly, and treatments received were also evaluated.

Statistical analysis

The data were analyzed using SPSS version 26.0 with a 95% confidence level. Frequency and percentage (n, %) were reported for categorical variables, whereas mean, standard deviation (mean±SD), minimum, maximum, and median (M) were reported for numerical variables.

For group comparisons, the independent samples t-test or Mann-Whitney U test was used for continuous variables, while the chi-square test was applied for associations between categorical variables. Logistic regression analysis was performed to identify factors associated with HFE mutation positivity. In addition, receiver operating characteristic (ROC) analysis was conducted to determine the cut-off values of hemoglobin, hematocrit, ferritin, and transferrin saturation (TS) for predicting HFE mutation status.

Results

Of 112 patients screened for HFE gene mutations, 106 were included in the analysis after exclusion criteria were applied. The cohort consisted of 77 males and 29 females (M/F ratio: 2.5/1), with a median age of 54 years (range, 19–83). Age at diagnosis was significantly higher in female patients compared with males ($p=0.002$). Overall, 44 patients (41.5%) were positive for an HFE mutation (31 males, 13 females), with no significant difference in prevalence by gender ($p=0.67$). The mean age did not differ between HFE-positive and HFE-negative patients ($p=0.23$); however, among mutation carriers, females had a significantly higher mean age at diagnosis than males ($p<0.001$).

Analysis of TS and serum ferritin levels showed that HFE mutation carriers had significantly higher mean TS compared

Table 1. Comparison of laboratory parameters by HFE mutation status

	HFE				p
	Negative		Positive		
	Min-max (M)	Mean±SD	Min-max (M)	Mean±SD	
Overall					
Age at diagnosis	20–83 (54)	52.76±15.41	19–77 (51.5)	49.34±16.63	0.268
Follow-up duration (months)	0–186.4 (18.3)	39.64±50.47	0.1–179.6 (31.5)	38.92±43.48	0.939
Platelets (10 ⁹ /L)	106–1067 (265.5)	368.57±231	123–1138 (284.5)	366.32±242.95	0.961
Leukocyte (10 ⁹ /L)	5.1–27.8 (7.9)	9.15±3.98	4–38.7 (7.6)	9.07±5.74	0.331
Neutrophil (10 ⁹ /L)	2.6–16.9 (4.7)	5.53±2.82	2.4–31.9 (4.2)	5.61±4.79	0.364
ALT	6–309 (21)	29.6±37.09	6–139 (21)	30.61±27.56	0.849
AST	8–220 (20)	25.63±26.98	9–101 (20.5)	24.86±17.99	0.964
Creatinine (mg/dL)	0.5–2.5 (0.9)	0.95±0.34	0.5–2.9 (0.9)	0.96±0.35	0.460
EPO (mU/mL)	1–25.9 (5.2)	7.14±6.31	1–46.8 (5.7)	8.84±9.77	0.517
TS (%)	18–83 (44.5)	44.96±15.21	22–91 (51.8)	51.41±16.49	0.040*
Female					
Serum Iron (µg/dL)	20–307 (94)	98.58±58.23	67–234 (108)	124±48.57	0.093
TIBC (µg/dL)	225–521 (337)	334.16±70.53	188–350 (296)	285.91±55.94	0.063
Hgb (g/dL)	9.5–19.9 (16.1)	15.18±3	8.5–16.7 (14.7)	14.27±2.4	0.380
Hct (%)	29.6–61.5 (47.6)	45.6±8.7	26.1–51.8 (43.4)	42.7±7.5	0.360
Ferritin (ng/mL)	23–1650 (79)	382.43±509.18	64–347 (143)	172.3±89.26	0.150
TS (%)	24.1±78.62 (39.5)	41.85±15.81	25.7–79.05 (48.4)	48.65±15.77	0.250
Male					
Serum Iron (µg/dL)	25–311 (108)	112.9±44.07	57–299 (127)	131.06±52.82	0.106
TIBC (µg/dL)	174–467 (315)	313.08±70.86	208–410 (321)	319.58±52.88	0.655
Hgb (g/dL)	10.4–19.5 (17.2)	16.77±1.9	12.7–18.8 (17)	16.73±1.5	0.920
Hct (%)	33.1–67 (50.2)	50.68±6.2	38–56.5 (50.4)	40.06±4.3	0.210
Ferritin (ng/mL)	18–2607 (131)	271.87±432.40	30–1127 (153)	250.48±244.57	0.810
TS (%)	18–83.2 (46.2)	46.04±15.02	18.9–81.7 (54.4)	52.56±16.9	0.080

Values are expressed as mean±SD. *: p<0.05 was considered statistically significant (t-test or Mann–Whitney test). Hgb, Hct, and iron-related parameters were evaluated separately by gender. HFE: Hemochromatosis protein; SD: Standard deviation; ALT: Alanine transaminase; AST: Aspartate transaminase; EPO: Erythropoietin; TIBC: Serum total iron-binding capacity; Hgb: Hemoglobin; Hct: Hematocrit; TS: Transferrin saturation.

with non-carriers (p=0.04). When evaluated separately by sex, neither TS nor ferritin levels differed significantly between HFE-positive and HFE-negative patients (p=0.81 and p=0.15 for ferritin in males and females, respectively; non-significant for TS). Other hematologic and biochemical parameters, including hemoglobin, hematocrit, leukocyte count, platelet count, ALT, and AST, did not show significant differences according to HFE mutation status (Table 1).

Of the total cohort, 56 patients (52.8%) had TS >45%, 13 (12.7%) had TS between 40–45%, and 13 (12.7%) presented with elevated serum ferritin for sex. Twenty-four patients (22.6%) underwent HFE testing due to clinical suspicion of hereditary hemochromatosis (HH). There was no significant association between the indications for testing and the presence of an HFE mutation, either overall or by sex (p=0.35).

Regarding underlying diagnoses, 37 patients (35%) were classified as CMPN (n=28, polycythemia vera [PV], 7 essential thrombocythemia [ET], 2 primary myelofibrosis [PMF]), 52

patients (49%) as secondary polycythemia, and 17 patients (16%) as non-polycythemic with elevated iron parameters. In addition, two patients had diffuse large B-cell lymphoma (DL-BCL), two had acute lymphoblastic leukemia (ALL), one had Hodgkin lymphoma (HL), and one had Ph-positive chronic myeloid leukemia (CML). Among comorbidities, hypertension was present in 30 (28.3%), diabetes mellitus in 19 (17.9%), and atherosclerotic cardiovascular disease in 13 (12.3%); other conditions were less frequent. For comparative analyses, patients were categorized into three groups: Group 1 included those with Myeloproliferative Neoplasms (MPNs; PV, ET, PMF), Group 2 comprised patients with secondary polycythemia, and Group 3 consisted of non-polycythemic patients presenting with elevated iron parameters.

Smoking status was available for all patients: 36 (34%) were current smokers, 16 (15.1%) were former smokers, and 54 (50.9%) never smoked. Including former smokers, half of the HFE-positive patients were ever-smokers, with no significant

Table 2. Transferrin saturation (TS, %) by clinical group and HFE status

A. Overall (all patients)		Mean±SD
Group		
Group 1 (CMPN)		52.7±14.2
Group 2 (Secondary polycythemia)		43.9±17.3
Group 3 (Non-polycythemic, elevated iron parameters)		50.8±15.2
Pairwise p: G1 vs G2=0.032*; G1 vs G3=0.86; G2 vs G3=0.12		
B. HFE-positive		
Group		
Group 1		53.8±14.2
Group 2		47.0±17.5
Group 3		63.9±10.4
Pairwise p: G1 vs G2=0.32; G1 vs G3=0.084; G2 vs G3=0.023* Groups 1+2 (all polycythemic [†]) vs Group 3: 48.1±16.9 vs 60.8±12.9 → p=0.026*		
C. HFE-negative		
Group		
Group 1		50.7±12.7
Group 2		41.2±17.0
Group 3		43.7±12.7
Pairwise p: G1 vs G2=0.036*; G1 vs G3=0.13; G2 vs G3=0.63 Groups 1+2 (all polycythemic [†]) vs Group 3: 44.22±16.0 vs 47.22±12.3 → p=0.52		

*: Values are mean±SD. Pairwise p values were calculated using t-test or Mann-Whitney test, as appropriate. p<0.05 was considered statistically significant. †: All polycythemic cases from Group 1 combined with all patients in Group 2.

Table 3. Distribution of HFE mutation genotypes across groups

HFE mutation type	Genotype	Group 1 (CMPN)	Group 2 (Secondary polycythemia)	Group 3 (Others [‡])	Total (%)	p
C282Y	Homozygous	0	1	0	1 (0.9%)	0.82
	Heterozygous	1	1	1	3 (2.8%) [†]	
	Negative	33	50	19	102 (96.2%)	
H63D	Homozygous	1	1	1	3 (2.8%)	0.65
	Heterozygous	11	22	5	38 (35.8%) [†]	
	Negative	22	27	15	64 (60.4%)	
	No result	-	1	-	1 (0.9%)	

†: Others: Non-polycythemic, elevated iron parameters; ‡: Compound C282Y and H63D mutation in one patient.

difference compared with HFE-negative patients (p=0.74). In Group 2 (secondary polycythemia), 22 patients (42%) were current smokers, while 7 (14%) had quit and 23 (44%) had never smoked. Notably, there were significantly more male patients in Group 2 compared with the other groups (p=0.014). Similarly, both HFE-positive and HFE-negative males were more common in Group 2 than in Groups 1 and 3 (p=0.031).

Group comparisons of TS (%) levels are summarized in Table 2. Patients with CMPN (Group 1) had significantly higher mean TS compared with those with secondary polycythemia (Group 2, p=0.032), while Group 3 values did not differ significantly from either group. In HFE-positive patients, TS was significant-

ly higher in Group 3 compared with Group 2 (p=0.023), but there was no difference between Groups 1 and 2. Moreover, non-polycythemic HFE-positive patients had higher mean TS compared with polycythemic HFE-positive patients (p=0.026). Among HFE-negative patients, TS was higher in Group 1 than in Group 2 (p=0.036). Finally, within Group 3, HFE-positive patients had significantly higher TS compared with HFE-negative patients (p=0.002).

The distribution of HFE genotypes is presented in Table 3. Overall, the C282Y and H63D variants were detected in 4 (3.8%) and 41 (38.7%) patients, respectively, with one patient harboring a compound heterozygous genotype. There

Table 4. Clinical characteristics of patients with heterozygous/homozygous C282Y, compound C282Y/H63D, and homozygous H63D mutations

ID	Sex	Age (y)	Genotype	Dx/Notes	Hgb (g/dL)	Hct (%)	TS (%)	Ferritin (ng/mL)	FU (month)	Status
1 (GK)	M	45	C282Y homozygous	Sec. polycythemia; β -thal trait; NS	16.2	50.5	71	735	31	Alive
2 (AA)	M	35	C282Y/H63D compound het.	Sec. polycythemia; NS	17.1	47.9	47.9	241	55	Alive
3 (SB)	F	72	C282Y heterozygous	MPN-ET; DM; CKD	13.8	42.5	33.2	347	33	Alive
4 (SD)	F	77	C282Y heterozygous	MPN-PV; JAK2 V617F+; DM	15.2	46.8	48	64	34	Alive
5 (AÇ)	M	49	H63D homozygous	HT; splenomegaly	13.7	41.6	76	1127	3	Alive
6 (KA)	M	54	H63D homozygous	Renal cyst; BPH; HLD	14.3	40.6	60	489	6	Alive
7 (ÖA)	M	31	H63D homozygous	Sec. polycythemia; S	17.7	48.1	53.7	48	2	Alive

M: Male; F: Female; MPN: Myeloproliferative neoplasm; PV: Polycythemia vera; ET: Essential thrombocythemia; DM: Diabetes mellitus; CKD: Chronic kidney disease; HT: hypertension; HLD: Hyperlipidemia; BPH: Benign prostatic hyperplasia; TS: Transferrin saturation; FU: Follow-up; S: Smoker; NS: Non-smoker; het.: Heterozygous.

was no significant difference in the distribution of homozygous and heterozygous forms of either genotype across the groups. The heterozygous H63D mutation was the most common finding, detected in 38 patients (35.8%), with similar prevalence among groups.

Evaluation of genotypes according to the clinical indications for HFE testing showed that homozygous C282Y was observed in one patient with TS >45%, and homozygous H63D in three patients. A heterozygous C282Y variant was identified in one patient with elevated ferritin, and compound C282Y/H63D heterozygosity was found in one patient with TS >45%. The heterozygous H63D variant was represented across all testing indications. No significant association was found between genotype type and testing indication ($p=0.78$ for C282Y, $p=0.45$ for H63D).

Clinical characteristics of patients with heterozygous or homozygous C282Y and homozygous H63D mutations are shown in Table 4. Among heterozygous H63D carriers, no significant differences were observed in hemoglobin, hematocrit, serum ferritin, or TS levels compared with HFE-negative patients in both PV and secondary polycythemia subgroups. The only significant difference was seen in Group 3, where TS was higher in heterozygous H63D carriers compared with non-carriers (Table 5). In subgroup analyses, ferritin levels appeared paradoxically higher in some HFE wild-type patients compared with mutation carriers (Table 5). Although such a finding is not consistent with the expected pathophysiology, ferritin is an acute phase reactant and may be elevated in the context of occult inflammatory, immunologic, or metabolic conditions. Despite the exclusion of patients with overt infection or inflammatory disease at baseline, comorbidities such as diabetes mellitus, thalassemia minor, or yet undiagnosed immunologic/rheumatologic disorders could have contributed to this observation.

ROC analysis identified a TS cut-off of 51.7% for predicting polycythemia in HFE-positive patients. Figures 1 and 2 pres-

ent the ROC curves with AUC, sensitivity, specificity, and 95% confidence intervals, but this threshold had limited diagnostic accuracy and was not clinically relevant.

Discussion

HFE hemochromatosis (type I hereditary hemochromatosis) represents the most common form of inherited iron overload. Polycythemia is defined as hemoglobin levels >16.5 g/dL in men and >16.0 g/dL in women and/or hematocrit levels >49% in men and >48% in women [4]. Previous studies have investigated the relationship between polycythemia and hereditary hemochromatosis. In one report, C282Y homozygous patients ($n=60$) exhibited significantly higher mean hemoglobin, hematocrit, MCV, and MCH values compared to 65 healthy controls without HFE mutations [8]. Similarly, in a series of 152 hereditary hemochromatosis patients (63.2% male), 44 (28.9%) carried the C282Y homozygous genotype, 10 (6.6%) the H63D homozygous genotype, and 27 (17.8%) the compound heterozygous C282Y/H63D genotype. Median hemoglobin and hematocrit values were 15.5 g/dL and 44.9% in C282Y homozygotes, 16.0 g/dL and 47% in H63D homozygotes, 15.8 g/dL and 46% in compound heterozygotes, 16.0 g/dL and 47% in C282Y heterozygotes, and 16.6 g/dL and 48% in H63D heterozygotes [9].

A retrospective analysis of 213 patients with hereditary hemochromatosis (mean age 53.6 ± 15.2 years; 143 males, 67.1%) identified HFE mutations in all cases. Homozygous C282Y mutations were present in 108 patients (50.7%), while polycythemia was observed in 59 patients (27.6%) [10]. In a large population-based study of 10,198 Caucasians, the reported allele frequencies were 0.63% for C282Y and 1.52% for H63D. Notably, mean hemoglobin and MCV levels were significantly higher in mutation carriers compared with non-carriers [11].

In our retrospective cohort of 106 patients screened for HFE mutations, 77 (73%) were male. A total of 44 patients (41.5%)

Table 5. Laboratory parameters of patients carrying the H63D heterozygous mutation compared with HFE wild-type patients

Parameter	PV (Group 1 subset)	Group 1 (MPNs)	Group 2 (Secondary polycythemia)	Group 3 (Others [†])
Hemoglobin (Male, g/dL)	H63D(+): 16.9±0.73 WT: 17.52±0.79 p=0.22	H63D(+): 15.85±1.8 WT: 17.0±2.0 p=0.24	H63D(+): 17.58±0.70 WT: 17.49±0.66 p=0.69	H63D(+): 14.3±1.2 WT: 13.68±2.3 p=0.64
Hematocrit (Male, %)	H63D(+): 50.9±1.57 WT: 53.52±4.59 p=0.28	H63D(+): 47.8±5.4 WT: 52.0±7.0 p=0.20	H63D(+): 51.23±2.59 WT: 51.0±2.29 p=0.76	H63D(+): 42.8±3.9 WT: 41.8±6.2 p=0.77
Hemoglobin (Female, g/dL)	H63D(+): 15.7±1.1 WT: 17.3±1.6 p=0.15	H63D(+): 13.3±2.8 WT: 16.3±3.1 p=0.079	H63D(+): 16.3±0.17 WT: 17.1±1.45 p=0.37	H63D(+): 12.1±2.41 WT: 11.5±1.5 p=0.66
Hematocrit (Female, %)	H63D(+): 47.5±4.97 WT: 51.9±5.95 p=0.29	H63D(+): 40.0±9.0 WT: 49.1±9.6 p=0.084	H63D(+): 48.1±3.18 WT: 51.08±5.25 p=0.40	H63D(+): 35.9±6.8 WT: 35.5±4.7 p=0.91
Ferritin (Male, ng/mL)	H63D(+): 168±149.4 WT: 114.39±57 p=0.28	H63D(+): 198±169.2 WT: 170.1±45.4 p=0.63	H63D(+): 174.4±124.1 WT: 297.93±545 p=0.36	H63D(+): 329±219.6 WT: 465.3±467.9 p=0.64
Ferritin (Female, ng/mL)	H63D(+): 146±2.64 WT: 147.1±154.8 p=0.96	H63D(+): 152.8±39 WT: 137.1±144.0 p=0.78	H63D(+): 225.3±112.1 WT: 146±2.64 p=0.28	H63D(+): 143±57.84 WT: 897.5±556.1 p=0.015*
Transferrin saturation (%)	H63D(+): 50.64±14.53 WT: 48.8±13.46 p=0.76	H63D(+): 54.13±11.9 WT: 49.8±13.1 p=0.33	H63D(+): 45.5±17.8 WT: 41.42±17.3 p=0.42	H63D(+): 70.3±11.5 WT: 45.19±12.1 p=0.007*

*: p<0.05; WT: HFE wild-type; †: Others: Non-polycythemic, elevated iron parameters; MPNs: Myeloproliferative Neoplasms.

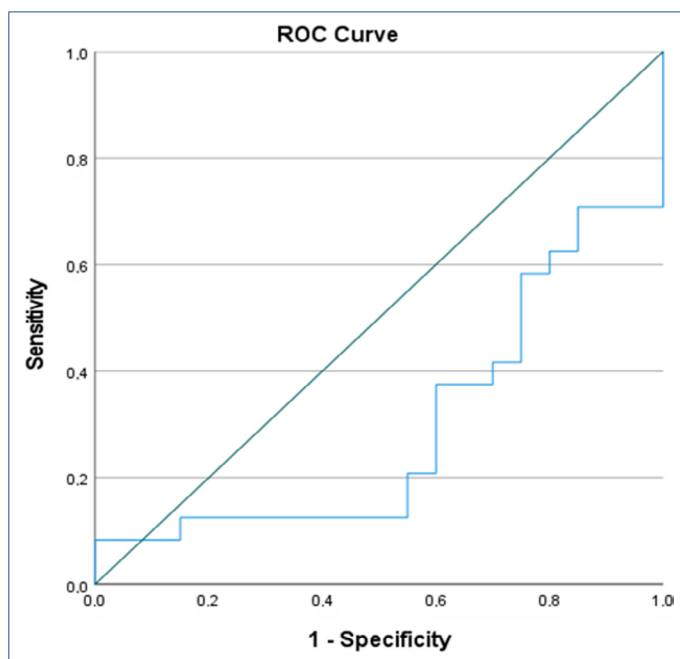


Figure 1. ROC curve for transferrin saturation (ST) in predicting HFE mutation positivity (Secondary polycythemia vs Myeloproliferative Neoplasms (MPNs)+Others). Receiver operating characteristic (ROC) curve for ST in predicting HFE mutation positivity in secondary polycythemia versus CMPN+Others. AUC=0.298 (95% CI: 0.140–0.456), p=0.02. Sensitivity/specificity were 37%/40% at the 51.7% cut-off, and 58%/25% at the 45.3% cut-off.

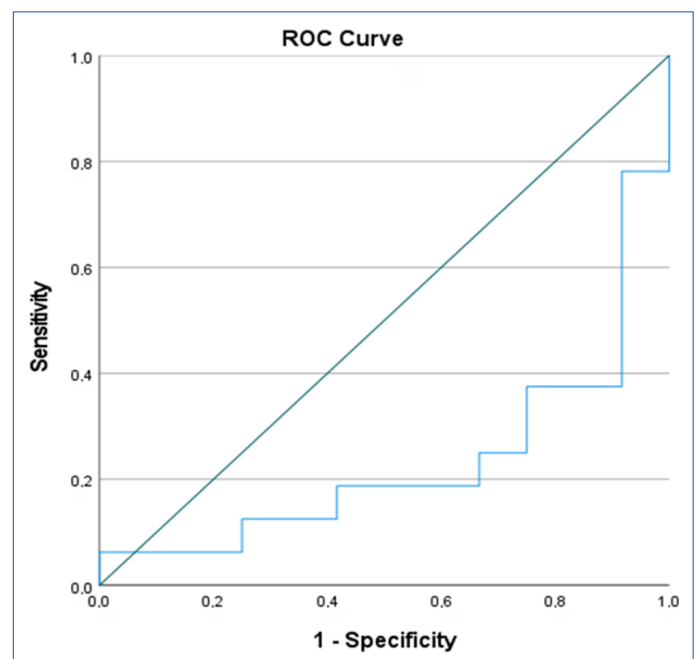


Figure 2. ROC curve for transferrin saturation (ST) in predicting HFE mutation positivity (Secondary polycythemia vs Myeloproliferative Neoplasms (MPNs)+Others). Receiver operating characteristic (ROC) curve for in predicting HFE mutation positivity in all polycythemias (including PV) versus non-polycythemias. AUC=0.232 (95% CI: 0.081–0.383), p=0.07. Sensitivity/specificity were 37%/25% at the 51.7% cut-off, and 56%/8% at the 45.3% cut-off.

tested positive for an HFE mutation (31 males, 13 females). The distribution of mutation positivity did not differ significantly between sexes ($p=0.67$). Although the absolute number of male carriers was more than twice that of females, this finding reflects the higher proportion of males in the cohort rather than a true male predominance in HFE mutation frequency.

There are only a limited number of studies on HFE gene mutations reported from our country. Most of these have identified the H63D variant, while the C282Y mutation has rarely been detected [12–17]. A large familial cluster carrying HFE mutations was described in the Diyarbakir region, where, among the relatives of a proband with a homozygous C282Y mutation, 17 were heterozygous for C282Y, 4 were heterozygous for H63D, and 2 carried a homozygous C282Y genotype [18]. In another national study that compared 86 patients with transferrin saturation (TS) above 45% to 57 controls with TS below 45%, no C282Y mutations were detected, while the H63D mutation was present in 27% of patients and 21% of controls, showing no significant association with the clinical phenotype [12]. More recently, a study published in 2021 investigated the frequency and distribution of HH-related mutations among 97 patients with elevated TS who were clinically suspected to have HH. Heterozygous H63D mutation was detected in 19 patients, 14 (20%) of whom had TS between 38–45% and 5 (18.5%) with TS >45% [17].

With the advent of genetic testing, the average age at diagnosis of HH has been reported to be similar in men and women, although disease manifestations tend to be milder in women [18]. In a large 12-year cohort of homozygous C282Y patients, clinical iron overload was observed in 28.4% of men but in only 1.2% of women [18]. In our study, the median age of patients with HFE mutations was in the fifth decade (51.5 years), consistent with previous reports. A significant age difference was observed between male and female carriers (median 45 years vs. 62 years, $p=0.001$). This may be attributed to the protective effect of menstruation, pregnancy, and childbirth, which tend to delay iron accumulation in women until the postmenopausal period.

When analyzed separately by sex, there was no significant difference in mean hemoglobin concentration or hematocrit levels between HFE mutation carriers and non-carriers ($p=0.92$ and 0.21 in males; $p=0.38$ and 0.36 in females). The predominance of heterozygous H63D carriers, along with only a single homozygous C282Y patient in our cohort, may partly explain these findings. We did not evaluate MCV in our study, as a substantial proportion of CMPN patients were receiving cytoreductive therapy such as hydroxyurea, and others were undergoing chemotherapy or radiotherapy for hematologic or solid malignancies, which could have confounded MCV measurements.

Initially, no relationship was thought to exist between polycythemia vera (PV) and hereditary hemochromatosis (HH). In 2002, a study screening 232 patients with various hematologic disorders for C282Y and H63D mutations found no significant association with PV [19]. Similarly, a 2004 study of 52 PV

patients reported no evidence of a link with HFE mutations [5]. However, subsequent reports have suggested that coexistence may occur in rare cases. In 2016, a case report described a 75-year-old woman with concurrent HH and PV [6]. More recently, in 2021, a heterozygous C282Y mutation was identified in a 59-year-old PV patient presenting with elevated serum ferritin and transferrin saturation [18]. Although HH and PV generally appear to be unrelated conditions, these reports indicate that overlap is possible, and some authors recommend considering HH screening in PV patients, and vice versa.

In our retrospective series, one noteworthy case was a 77-year-old female patient who had been diagnosed with PV three years earlier. She was JAK2-positive, had low serum erythropoietin levels, and a history of type 2 diabetes mellitus. The patient was receiving hydroxyurea and acetylsalicylic acid (ASA) as part of her PV management. Iron metabolism in PV can be influenced by abnormal erythropoiesis, systemic inflammation, reduced iron availability, and hypoxia-driven regulation of intestinal iron absorption [18]. Moreover, gastrointestinal lesions such as erosions, ulcers, and *Helicobacter pylori* infection are reported more frequently in PV compared with the general population [18]. Most patients with PV exhibit iron deficiency at diagnosis, even prior to initiation of therapeutic phlebotomy, the standard treatment approach [20, 21]. Although thrombosis is the most serious complication in PV, bleeding also represents a clinically important risk. At diagnosis, the reported rates of major thrombosis and major bleeding were 24.3% and 4.3%, respectively, whereas at follow-up under cytoreductive therapy and ASA, these rates were 18.4% and 1.8% [22]. Interestingly, despite long-term ASA use and gastrointestinal complaints, this patient exhibited persistently elevated transferrin saturation (48%). HFE mutation analysis revealed heterozygous C282Y positivity.

Another case of interest in our cohort was a 72-year-old female patient with essential thrombocythemia (ET), also diagnosed with type 2 diabetes mellitus and chronic renal failure. She was negative for JAK2 V617F mutation but was receiving hydroxyurea and ASA. Although her transferrin saturation was 33%, her serum ferritin level was elevated in the absence of an inflammatory condition, with C-reactive protein values within the normal range. Genetic testing for HFE mutations was therefore performed, revealing heterozygous C282Y positivity.

In our study, two male patients carrying a homozygous C282Y mutation and one patient with a compound C282Y/H63D heterozygous mutation presented with secondary polycythemia. One of the C282Y homozygous patients was also a carrier of β -thalassemia. Despite a hemoglobin level of 16.2 g/dL, his hematocrit was 50.5%, leading to the diagnosis of secondary polycythemia. Leukocyte and platelet counts were within normal limits. Neither of the patients had a history of smoking, and no alternative cause for secondary polycythemia was identified. Additionally, a 31-year-old male patient with homozygous H63D mutation and a histo-

ry of smoking developed secondary polycythemia and required therapeutic phlebotomy. Asif et al. [9] reported that although previous studies primarily associated elevated hemoglobin levels with homozygous C282Y patients, their own findings also demonstrated increased hemoglobin levels in heterozygous carriers [11].

In a cohort of 152 patients with HFE mutations, polycythemia was observed in 33 individuals (27.7%). The authors argued that even carrier status for the HFE mutation could be associated with elevated hemoglobin and hematocrit levels, independent of serum ferritin concentration and secondary causes of polycythemia. In contrast, Khan et al. [10] reported a different perspective. Among 213 patients with HFE mutations, polycythemia was detected in 23 (10.8%) and 59 (27.6%) cases, respectively. They concluded that although hereditary hemochromatosis may confer relative protection against anemia, polycythemia does not develop in the majority of patients, and the limited available data on hemoglobin parameters do not support a high overall prevalence of polycythemia in this population. In our view, the polycythemia rates reported by the two groups appear broadly comparable.

In our study, secondary polycythemia was identified in three of seven patients with clinically significant C282Y or homozygous H63D mutations. In two of these cases, no secondary cause of polycythemia could be determined. Among the remaining patients with secondary polycythemia, heterozygous H63D mutations were detected in 21 individuals (18 males, 3 females). Overall, 24 of 106 patients screened for HFE mutations (23%) had secondary polycythemia associated with an HFE mutation. When considered in relation to mutation status, 24 of 44 patients with HFE mutations (55%) also had secondary polycythemia. We did not observe a significant difference in the distribution of patients with or without HFE mutations across the groups. It should be noted, however, that secondary polycythemia accounted for the majority of patients who underwent genetic testing due to transferrin saturation >40%, elevated ferritin relative to sex, or a clinical suspicion of HH, even though the absolute numbers were limited.

Within the secondary polycythemia group, 13 of 24 patients with HFE mutations and 16 of 28 patients without HFE mutations (including one case with undetectable H63D mutation) reported a history of smoking, with no significant difference between them ($p=0.26$). This finding suggests that smoking, although a well-established cause of secondary polycythemia, is not sufficient to exclude the presence of an HFE mutation. Moreover, the mean transferrin saturation in Group 2 patients with secondary polycythemia was significantly lower than in Group 3 patients without polycythemia ($47.0\pm 17.5\%$ vs. $63.9\pm 10.4\%$, $p=0.023$). ROC analysis indicated a TS cut-off value of 51.7% for differentiating patients with and without HFE mutations and polycythemia. However, since current recommendations already use TS >45% as the threshold for HFE testing, this higher cut-off was not considered clinically useful.

Consistent with this, recent clinical guidelines for hereditary hemochromatosis emphasize that TS values above 45–50% are more diagnostically informative than hyperferritinemia [23]. In line with these recommendations, our analysis highlighted TS as a more meaningful parameter than serum ferritin when evaluating the relationship between HFE genotypes and polycythemia. While the most frequent genotypes associated with iron overload are C282Y homozygosity and C282Y/H63D compound heterozygosity, several reports have also documented iron overload and even polycythemia in patients with H63D heterozygosity. A recent case report described a patient carrying an H63D heterozygous variant who presented with iron overload and erythrocytosis [24]. Similarly, Sandnes et al. [25] demonstrated that patients with H63D heterozygosity (H63D/WT) had a median ferritin level of 711 ng/mL—comparable to homozygous variants—with ferritin >500 ng/mL observed in 91.3% and TSAT>45% in 17.4% of cases. These findings collectively suggest that H63D heterozygosity, although often considered clinically less relevant, may still contribute to increased iron burden and polycythemia in certain individuals.

Limitations

The clinical relevance of C282Y and homozygous H63D mutations in HH is well recognized. However, the relatively small number of patients carrying these mutations, as well as the overall limited number of HFE-positive cases compared with previous studies, represents a limitation of our study. Larger, prospective studies are warranted to better define these associations.

Conclusion

In this retrospective study, we demonstrated that patients with HFE mutations and polycythemia—particularly those with secondary polycythemia—exhibited significantly lower mean TS levels compared to mutation-positive patients without polycythemia. Importantly, our findings suggest that in polycythemic patients, iron parameters may appear deceptively low, likely due to increased erythrocyte mass consuming or “diluting” available iron. This phenomenon may mask underlying HFE positivity, meaning that HFE mutations can be present even at lower-than-expected TS values.

Secondary polycythemia is a common clinical condition, yet in many cases the underlying cause remains unexplained. In our cohort, the frequency of HFE positivity among patients with secondary polycythemia was relatively high, even though the difference across groups did not reach statistical significance. Taken together, these findings emphasize that measurement of TS and serum ferritin should not be overlooked in patients with secondary polycythemia, and that HFE genotyping may be considered even at lower TS thresholds when the etiology of polycythemia remains unclear. Recognizing this association is clinically relevant for patient management, particularly in guiding decisions about therapeutic phlebotomy.

Disclosures

Ethics Committee Approval: The study was approved by the Izmir Katip Çelebi University Atatürk Training and Research Hospital Clinical Research Ethics Committee (no: 0054, date: 21/03/2023).

Informed Consent: Informed consent was obtained from all participants.

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