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Title

Hemochromatosis gene mutations may affect the survival of patients with myelodysplastic syndrome

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Keywords

Myelodysplastic syndrome; *HFE*; C282Y; H63D; Iron metabolism; Overall Survival

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Abstract

Objectives: The recent availability of potent oral iron chelators is renewing an interest in the assessment of the possible impact of *HFE* genetics in MDS**.**

Methods: 36 newly diagnosed patients with MDS were studied for parameters of iron metabolism in addition to C282Y and H63D mutations of the *HFE* gene.

Results: Mutations were present in 11 out of 36 patients (31%), which was not different from our general population and were equally distributed among MDS subtypes. Mutated patients had higher ferritin levels (p=0.039) and lower TIBC (p=0.018). Ferritin was found to be higher for the untransfused mutated patients (p=0.017), but not for transfusion-dependent patients in whom ferritin levels correlated significantly with the number of blood units received (p=0.04). There was no difference in the number of blood units received between the mutated and wild type patients. A new observation made was that the mutated patients had a lower overall survival (OS) in addition to a poorer leukemia free survival (LFS) (p=0.004 and p=0.003, respectively).

Discussion: The *HFE* gene mutations are not more frequent in MDS patients. Iron overload in mutated patients was higher but there was no correlation found using supportive therapy for anaemia. The effect of mutations on survival could be mediated by changes in iron metabolism. **Conclusion:** The *HFE* gentopye may predict MDS prognosis and there is a need for further studies. It remains a challenging question if *HFE* mutated MDS patients should be considered for potent iron chelation therapy.

Keywords

Myelodysplastic syndrome; *HFE*; C282Y; H63D; Iron metabolism; Overall Survival

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematopoietic stem cell malignancies characterized by ineffective hematopoiesis, progressive cytopenias and high risk of transformation to acute leukemia. These patients are at risk of developing an iron-replete state and hemochromatosis due to frequent transfusion dependency and characteristics of the disease itself.[1] Transfusion dependency has been recognized as an independent prognostic factor for the survival of patients with MDS[2] and iron chelation therapy leads to the improvement of survival in these patients.[3-5]

Increased frequency of two hemochromatosis (*HFE*) gene mutations, C282Y and H63D, among MDS patients was reported by Varkony et al.[6] This finding was confirmed only for a subset of MDS patients with RARS[7] and could not be confirmed by other author groups for MDS patients in general.[7-9] Differences were attributed to a different geographic distribution of the *HFE* gene mutations among populations.

No significant differences in serum ferritin concentration between mutated and wild type MDS patients were reported, in due to a small number of patients. It was shown however, that iron overload could develop with a lower number of transfusion units in mutated MDS patients.[6] These findings are consistent with five large population studies whereby heterozygosity for *HFE* mutations was associated with subtle changes in iron metabolism (higher transferrin saturation) but not with higher serum ferritin concentrations.[10-14]

In this study, we present the functional impact of *HFE* gene mutations on iron metabolism and survival of MDS patients.

Patients

We studied a group of 36 newly diagnosed patients with MDS, diagnosed in period from 2008 to 2014. Of this group, 53% of the patients were male, median age at diagnosis was 74 years of age (53-89). Among our patients 13 (36%) were diagnosed with refractory anemia (RA), 12 (33%) with refractory anemia with ring sideroblasts (RARS), seven (19%) with refractory anemia with excess of blasts (RAEB), two with RAEB1 and five with RAEB2, three (8%) with unclassified MDS and one

with 5q- syndrome. Patients with acute leukemia and signs of myelodysplasia at time of diagnosis were not included in this study.

The study protocol was approved by the University Hospital Dubrava's review board. All subjects involved in this study provided written informed consent.

Methods

Parameters of iron metabolism (Fe, TIBC, UIBC and ferritin) were measured repeatedly during the follow-up period and the most recent follow up data was used.

Genomic DNA was extracted (QIAamp® DNA Blood mini kit) from 3 mL of EDTA-anticoagulated blood. G-to-A transition for C282Y and C-to-G substitution for H63D mutation of *HFE* gene were detected by AS PCR in an ABI Prism 7300 Sequence Detection system (Applied Biosystems).

Frequencies of categorical variables were compared using the Fisher exact test or Chi Squared test where appropriate. Numerical variables were compared using Mann-Whitney U test. The Spearman rank correlation was used to assess the correlation between variables. Survival analysis was performed using methods of Kaplan and Meier and the log-rank test.[15] All statistical tests were two-sided and P values <0.05 were considered significant. Due to a small number of patients, we analyzed all patients with mutated *HFE* gene (C282Y and H63D mutations) as one group in comparison to wild type patients.

Results

HFE mutations frequency

HFE gene mutations were present in 11 out of 36 patients (31%). The C282Y mutation was present in three patients (8%), one patient was homozygous and the other two were heterozygote carriers. The H63D mutation was present in 9 patients (25%), all were heterozygous carriers. One patient was a compound heterozygote harboring both mutations. The *HFE* gene was mutated in 4 out of 13 patients with RA (31%), 4 out of 12 patients with RARS (34%), 3 out of 7 patients with RAEB (43%) and no mutations were present in the other 4 patients (Table 1).

The reported prevalence of *HFE* gene mutations in the Croatian population is 32%, 6.5% and 26.5% for C282Y and H63D respectively.[16] When compared, our patients' mutated frequency did not differ

significantly (p=0.864, p=0.716 and p=0.851 for mutated *HFE*, C282Y and H63D respectively) (Table 2). There were no significant differences in age at diagnosis, sex or need for blood transfusions between the mutated and wild type patients.

Parameters of iron metabolism

Increased values of transferrin bound iron, transferrin saturation and ferritin and decreased values of TIBC were observed in the mutated group of patients when compared to the wild type patients (Table 3). We observed a significant difference in the frequency of patients who developed iron overload (measured as ferritin over 1000 mcg/l) which was more prevalent in mutated group of patients (67% vs. 25%, p=0.044). We also observed a statistical significance for ferritin values (median 1113 mcg/l vs. 458 mcg/l, p=0.039) and TIBC values (median 39.4 mcmol/l vs. 50.7 mcmol/l, p=0.018). The effect on ferritin concentrations was present and statistically significant in a subgroup of non-transfused patients (p=0.017), but without any statistical significance between the mutated and the wild type patients in the subgroup of transfusion dependent patients. Ferritin levels correlated significantly with number of blood units received (Spearman Rho=0.52, p=0.04) and there was no significant difference in number of blood units received between mutated and wild type patients (median 54 vs. 57.5, p=0.562).

Survival analysis by HFE status

We observed a statistically significant effect of *HFE* gene mutations on the overall survival (OS) of MDS patients (p=0.004) (Figure 1 A). The overall survival at 36 months was 23% in the mutated group vs. 87% in wild type patients with a hazard ratio of 13.3. The median follow-up was 29 months. A statistical significant effect was also observed on leukemia free survival (LFS, p=0.003) (Figure 1 B). With the exclusion of patients with RAEB, significant changes in OS and LFS were still present (a total of 29 patients, p=0.003 and p=0.004 respectively). In addition, by excluding C282Y homozygous and C282Y/H63D compound heterozygous patients the study results were still significant (a total of 34 heterozygous patients, p=0.032 and p=0.028 for OS and LFS respectively).

Discussion

In our study, *HFE* gene mutations were as frequent among MDS patients as within the healthy population. In addition, we did not observe a significant increase in the frequency of *HFE* gene mutations in the RARS subgroup of patients, although such subgroup analysis is difficult to interpret based on the small study number. Noted differences among previous reports are at least in part mediated by a different geographical distribution of *HFE* gene mutations, but may also be affected by the mode of patient referral and the type of methodology used (exclusion of MDS in transformation patients, study design).

HFE mutational status undoubtedly affects iron metabolism, especially with the high risk genotype that can lead to hereditary hemochromatosis (homozygosity for C282Y).[17, 18] When excessive body iron saturates reusable ferritin storage, it is deposited in form of water-insoluble hemosiderin which can be an initiator of reactive oxygen species[19, 20] and lead to adjacent tissue damage and malignant transformation. It is this mechanism that has been implicated in development of hepatocellular carcinoma and other malignancies in patients with hereditary hemochromatosis[21, 22] and is also suspected to be a possible mechanism of defective hematopoiesis in some MDS patients.[7, 23] Single heterozygosity for these mutations does not lead to overt iron accumulation as shown by the aforementioned population studies.[10-14] As our results show (mainly heterozygote patients, one C282Y homozygote, one C282Y/H63D compound heterozygote), mutations in *HFE* gene have a profound effect on ferritin and TIBC values in myelodysplastic syndrome. The effect on ferritin is present in a subgroup of non-transfused patients, suggesting that the mutated *HFE* gene contributes to intrinsic characteristic of MDS to accumulate iron.

One of the most intriguing observations made in this study, not previously reported, is the effect of the mutated *HFE* gene on the overall survival and leukemia free survival in patients with MDS. These effects could be mediated by the aforementioned changes in iron metabolism. Both subclinical and manifest iron accumulation lead to an increase in the generation of free radicals and local tissue damage which in turn increases the chances of disease transformation to acute leukemia or death. The effect of *HFE* mutations on the survival of our patients cannot be attributed to transfusion dependency

as there are no significant differences in the number of blood units received or frequency of transfusion dependent patients between the mutated and the wild type group. In addition, the effect of the mutated *HFE* gene on survival is still present when patients with RAEB (entity with high risk for worst outcome) are excluded. The limitations of this study encompass the small number of subjects and the inability to obtain International Prognostic Scoring System (IPSS) score for all patients involved. The described findings are an interesting contribution into the pathogenesis of MDS and are worth further investigation in future studies, as these studies are necessary in determining whether or not *HFE* mutated MDS patients should be considered for the obligatory institution of potent iron chelation therapy.

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Table 1
HFE genotype by MDS subtype. Total number and number of heterozygous patients (unless specially noted) are provided; hom. - homozygote

MDS subtype	C282Y	H63D	Wild type
RA (13)	1	3	10
RARS (12)	0	4	8
RAEB (7)	1 C282Y/H63D, 1 C282Y hom.	1	4
Unclassified MDS and 5q- (4)	0	0	4

Table 2

Frequency of *HFE* mutations

	MDS patients	Controls[16]	p value
Both mutations	11/37 (30%)	64/200 (32%)	0.864
C282Y	3/37 (8%)	13/200 (7%)	0.716
H63D	9/37 (24%)	53/200 (27%)	0.851

Table 3

Parameters of iron metabolism (median values)

	***HFE* mutated patients**	***HFE* wild type patients**	**p value**
Fe	25.95 mcmol/l	18.9 mcmol/l	0.583
TIBC	39.5 mcmol/l	50.7 mcmol/l	0.018
Transferrin saturation	66%	37%	0.29
Ferritin	1113 mcg/l	458 mcg/l	0.039

Figure 1

A) Overall survival curves for the HFE wild type and mutated patients (p=0.004);

B) Leukemia free survival curves for the HFE wild type and mutated patients (p=0.003)