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ORIGINAL ARTICLE

JAK2 V617F mutation in myelodysplastic syndrome, myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable, refractory anemia with ring sideroblasts with thrombocytosis, and acute myeloid leukemia

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Background
The JAK2 V617F mutation has been noted in the cases of polycythemia vera, essential thrombocythemia, and primary myelofibrosis patients. This mutation occurs less frequently in acute myeloid leukemia (AML) and other hematologic diseases, such as myelodysplastic syndrome (MDS); myelodysplatic syndrome/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U); and refractory anemia with ring sideroblasts with thrombocytosis (RARS-T).

Methods
Patients diagnosed with hematologic diseases other than MPN who visited Seoul St Mary's Hospital from January 2007 to February 2010 were selected. A total of 43 patients were enrolled in this study: 12 MDS, 9 MDS/MPN-U, 7 RARS-T, and 15 AML patients. The diseases were diagnosed according to the 2008 WHO classification criteria. Data obtained from JAK2 V617F mutation analysis and cytogenetic study as well as complete blood count and clinical data were analyzed.

Results
Of the 43 patients, 6 (13.9%) harbored the JAK2 V617F mutation. The incidence of the JAK2 V617F mutation in each patient group was as follows: 8.3% (1/12), MDS; 22.2% (2/9), MDS/MPN-U; 14.3% (1/7), RARS-T; and 13.3%, (2/15) AML. The platelet count was higher than 450×10^9/L in 3 of the 6 patients (50%) harboring the JAK2 V617F mutation, and it was in the normal range in the remaining 3 patients. Among the 6 patients, 1 MDS and 1 MDS/MPN-U patients had the 46,XX,del(20)(q11.2) karyotype.

Conclusion
The JAK2 V617F mutation is associated with an increased platelet count in MDS, MDS/MPN-U, RARS-T, and AML patients. Cytogenetic abnormalities of del(20)(q11.2) occurred in 1/3 of patients with the JAK2 V617F mutation but further studies are required to confirm this association.

Key Words JAK2 V617F, MDS, MDS/MPN-U, RARS-T, AML

INTRODUCTION

A somatic mutation in JAK2 V617F in hematopoietic stem cells has been reported to cause increased sensitivity to erythropoietin and independent growth to growth factor [1]. The mutation is commonly found in a majority of patients with myeloproliferative neoplasm (MPN) characterized by proliferation of one or more of the myeloid cell lineages in the bone marrow and circulating immature cells in the peripheral blood. The mutation occurs in 95% of patients with polycythemia vera and 50% of patients with essential thrombocythemia and primary myelofibrosis and in other diseases

included in this category, except chronic myelogenous leukemia [2]. The JAK2 V617F mutation is uncommon in other disease category with a few reports of variable incidence; further, no reports of this mutation in Korean patients exist. In this study, the JAK2 V617F mutation was studied in patients with hematologic diseases other than MPN.

MATERIALS AND METHODS

1. Patients

In this study, we included patients who were examined for the JAK2 V617F mutation at Seoul St. Mary's Hospital from January 2007 to February 2010. Forty three patients were enrolled, and the patients were grouped on the basis of their diagnosis according to the 2008 WHO classification as follows [8]: 12, myelodysplastic syndrome (MDS); 9, myelodysplatic syndrome/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U); 7, refractory anemia with ring sideroblasts with thrombocytosis (RARS-T); 15, acute myeloid leukemia (AML). One case of acute panmyelosis with fibrosis (APF) was included in the AML group. Three refractory cytopenia with multilineage dysplasia (RCMD) patients, 4 refractory anemia with excess blasts (RAEB)-1 patients, and 5 RAEB-2 patients were included in the MDS group. In case of AML patients, the regimen for induction chemotherapy with idarubicin and behenoyl arabinofuranosylcytosine (BHAC) was administered and transplantation was performed as previously reported [3]. In the case of MDS patients, most of them were treated with azacitidine, and in the case of MDS/MPN-U patients, hydroxyurea was administered. The clinical characteristics and laboratory data including the karyotyping results of the patients are listed in Table 1.

2. JAK2 V617F mutation study

DNA was extracted from bone marrow aspiration samples with the QIAamp DNA Blood Mini Kit (Valencia, CA, USA) and quantified by spectrophotometry (NanoDrop Technologies Inc, Wilmington, DE). The JAK2 V617F study was performed by the melting curve analysis method. Melting curve analysis was performed using the Real-Q^{TM} JAK2 V617F detection kit (BioSewoom Inc, Seoul, Korea) and Hotstar *Taq* plus DNA polymerase (Qiagen, Valencia, CA.) in the Rotor gene 6000 (Valencia, CA, USA). A melting temperature of approximately 75±0.1°C was considered to be indicative of the presence of the JAK2 V617F mutation and a temperature over 76°C, indicative of the absence of the mutation (Fig. 1). In case of 4 RARS-T patients, JAK2 exon12 mutation analysis by the melting curve method was performed as sufficient DNA was obtained from these patients [4, 5].

3. Statistical analysis

Descriptive and statistically analyzed data were based on variables collected at the time of initial diagnosis. The Kruskal Wallis test was used to compare the 4 disease groups, and the Mann Whitney U-test was used in the case of variables that showed statistical significance against each group for that variable. Mann Whitney U-test was also used to compare the group with the JAK2 wild-type allele with the group containing the JAK2 V617F mutation. To determine the association between the JAK2 V617F mutation status and the

Table 1. Clinical characteristics and JAK2 V617F mutation status of patients based on the diagnosis.

Variable	MDS (n=12)	MDS/MPN-U (n=9)	RARST (n=7)	AML (n=15)
Age[a)]	67 (36-74)	65 (53-69)	(49-74)	40 (16-80)
Sex				
Men	7	6	3	9
Women	5	3	4	6
WBC (×10^9/L)	2.3 (0.9-30.9)	5.2 (2.1-21.9)	5.1 (4.9-839.5)	23.1 (0.9-139.6)
Hb (g/dL)	9.5 (6.0-11.0)	9.0 (7.9-11.4)	10.1 (7.0-12.0)	9.4 (5.0-12.2)
PLT (×10^9/L)	118 (11-362)	356 (63-1,250)	695 (519-1565)	110 (19-2,120)
MCV (fL)	90.7 (83.5-107.3)	90.5 (79.8-100.2)	102 (89.6-108.0)	91.4 (78.3-124.1)
MCH (pg)	30.9 (27.9-35.2)	28.5 (23.9-32.8)	34.6 (29.2-36.0)	30.8 (24.5-41.6)
MCHC (g/dL)	32.9 (30.3-35.9)	31.9 (28.9-34.3)	33.3 (32.6-35.8)	33.5 (28.9-36.7)
BM cellularity (%)	50 (10-100)	95 (50-100)	90 (40-100)	100 (50-100)
BM blast (%)	10 (0-15)	2.0 (0-9)	1 (1-3)	70 (21-98)
JAK2 V617F mutation (%)	1/12 (8.3)	2/9 (22.2)	1/7 (14.3)	2/15 (13.3)
Cytogenetics				
Normal	7	3	6	5
1-2 abnormalites	3	5	1	6
Complex karyotype	2	1	0	4
Survival (month)	11 (1-61)	12 (1-76)	30 (19-76)	15 (2-39)

[a)]All the continuous variables are presented as median (range).
Abbreviations: MDS, myelodysplastic syndrome; MDS/MPN-U, myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable; RARS-T, refractory anemia with ring sideroblasts with thrombocytosis; AML, acute myeloid leukemia; BM, bone marrow.

platelet count, linear by linear association was performed. All statistical analyses were performed using the Medcalc software 9.0 (Medcalc, Mariakerke, Belgium).

All the *P*-values were 2-tailed and statistical significance was set at the level of $P<0.05$. The overall survival (OS) was defined as the length of time from the date of diagnosis to the date of death caused by factors related to the diagnosed disease.

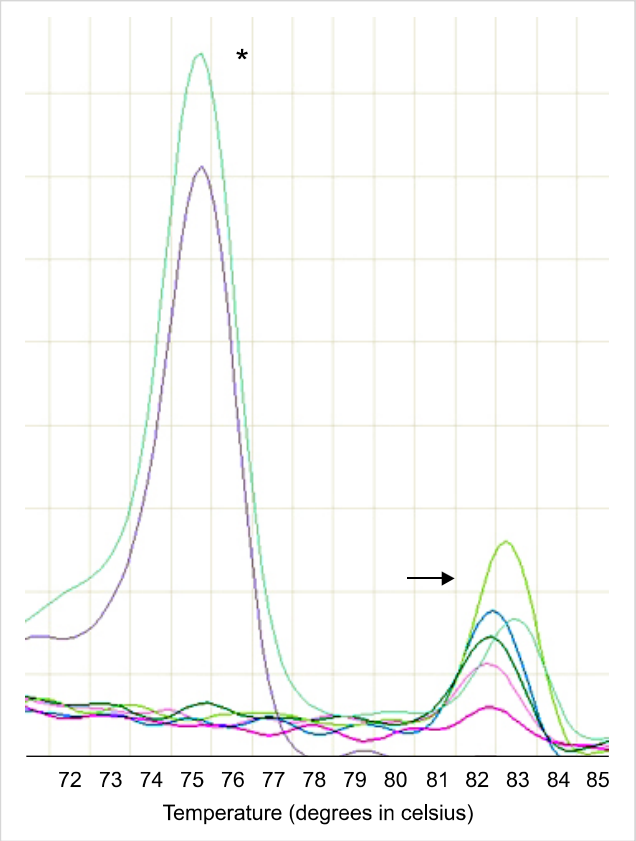

Fig. 1. JAK2 Exon14 (V617F) test performed by the melting curve analysis method. Analysis of positive control (purple) and positive V617F mutation in an RARS-T patient (green) with a melting curve at 75°C (indicated with star shape) and another 5 controls with wild-type alleles (arrow).

RESULTS

The JAK2 V617F mutation was identified in 6 of 43 (13.9%) patients. The incidence of the JAK2 V617F mutation in each diagnosis group was as follows: 8.3% (1/12), MDS; 22.2% (2/9), MDS/MPN-U; 14.3% (1/7), RARS-T; 13.3% (2/15), AML (Table 1). The JAK2 exon12 mutation study conducted in 4 RARS-T patients revealed that all of them harbored wild-type allele.

The platelet count of patients with the JAK2 V617F mutation was higher than 450×10^9/L in 3 of the 6 patients (50%), and it was within normal range in the remaining 3 patients (Table 2). The median platelet count in the JAK2 V617F mutation group was 532×10^9/L (range, 194×10^9/L to $1,562\times10^9$/L), this value is much higher than that in the case of the JAK2 wild-type group which had a median platelet count of 158×10^9/L (range, 11×10^9/L to $2,120\times10^9$/L) ($P<0.01$) (Table 3). In addition, linear by linear association analysis showed that the JAK2 V617F mutation and platelet count were positively correlated with a value of 3.831 ($P=0.05$).

Among the 6 patients with the JAK2 V617F mutation, 4 showed an abnormal karyotype. Two patients each in the MDS and MDS/MPN-U groups, respectively, had a karyotype of 46,XX,del(20)(q11.2). The karyotypes of the other 2 were 46,XX,del(10)(q22q26)[6]/46,XX[14] and 47,XX,+8 [18]/46,XX [2], respectively. There were 2 more MDS patients with the karyotype del(20)(q11.2) among the 37 patients with the JAK2 V617F wild-type allele. The other complete blood count (CBC) parameters, including the RBC count, Hb levels, WBC count, and RBC indices, were not significantly different between the JAK2 V617F mutation group and wild-type group.

DISCUSSION

The JAK2 V617F mutation has been reported to be strongly

Table 2. Clinical and laboratory data of JAK2 V617F-positive cases.

Age	Sex	Diagnosis	Cytogenetics	WBC ($\times10^9$/L)	Hb (g/dL)	PLT ($\times10^9$/L)	Cellularity (%)	Blast (%)	Treatment	Survival (mo)	Outcome
40	F	AML	46,XX,del(10)(q22q26)[6]/46,XX[14]	1.6	7	194	80	60	alloPBSCT	21	Alive
18	F	APF	47,XX,+8 [18]/46,XX[2]	2.9	10.1	703	100	21	uPBSCT	15	Alive
53	F	MDS/MPD-U	46,XX,del(20)(q11.2)	2.6	8.3	1,143	100	3	Azacitidine, alloPBSCT	76	Alive
66	F	MDS/MPN-U	46,XX	9.8	10.8	356	100	2	Hydroxyurea	FU loss	FU loss
72	F	RAEB	46,XY,del(20)(q11.2)	6.7	9.8	362	NA	NA	Conservative	61	Alive
68	M	RARS-T	46,XX	26.9	11.2	1,565	90	1	HydroxyureaAnagrelide	19	Alive

Abbreviations: AML, acute myeloid leukemia; APF, acute panmyelosis with fibrosis; MDS/MPN-U, myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable; RAEB, refractory anemia with excess blasts; RARS-T, refractory anemia with ring sideroblasts with thrombocytosis; alloPBSCT, allogenous peripheral blood stem cell transplantation; uPBSCT, unrelated donor peripheral blood stem cell transplantation; FU loss, follow up loss; NA, not available.

Table 3. Comparison of clinical characteristics and laboratory data of the JAK2 mutation group and the JAK2 wild-type group.

	JAK2 wild type (n=37)	JAK2 V617F (n=6)
Age[a]	58 (16-80)	59.5 (18-72)
Sex		
Male	24	1
Female	13	5
Diagnosis		
MDS	11	1
MDS/MPN-U	7	2
RARS-T	6	1
AML	13	2
Complete blood count[a]		
WBC ($\times 10^9$/L)	4.9 (0.9-139.6)	4.8 (1.6-26.9)
Hg (g/dL)	9.3 (5.0-12.3)	9.9 (7.0-11.2)
PLT ($\times 10^9$/L)	158 (11-2,120)	532 (194-1,562)
MCV (fL)	91.2 (78.3-108.0)	91.9 (81.6-124.1)
MCH (pg)	30.8 (24.5-36.0)	29.7 (23.9-41.6)
MCHC (g/dL)	33.1 (28.9-36.7)	31.3 (29.0-33.5)
Cytogenetics		
Normal	19	2
1-2 abnormalities	11	3
Complex karyotype	7	1
Survival (mo)	13 (1-75)	21 (15-76)

[a]All the continuous variables are presented as median (range)
Abbreviations: MDS, myelodysplastic syndrome; MDS/MPN-U, myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable; RARS-T, refractory anemia with ring sideroblasts with thrombocytosis; AML, acute myeloid leukemia.

associated with the pathogenesis of polycythemia vera. This mutation causes hyperproliferation of erythrocytes, granulocytes, and platelet precursors in the bone marrow in other types of MPNs also [6]. The incidence of this mutation is 6.7% in MDS [7], 40% in MDS/MPN-U [8], 53% in RARS-T [8], and 1.8% to 28.0% in AML [9-14].

In this study of Korean patients, the JAK2 V617F mutation was identified in 6 of 43 (13.9%) patients. The incidence of the JAK2 V617F mutation in each diagnosis group was as follows: 8.3%, MDS; 22.2%, MDS/MPN-U; 14.3%, RARS-T; and 13.3%, AML. The MDS/MPN-U cases account for 2% of all the cases classified as MDS [15], and the incidence of the JAK2 mutation in this study was 22.2% (2/9) compared to the 40% (2/5) reported in literature [8]. Few reports of MDS/MPN-U along with the JAK2 V617F mutation exist, and further studies are required to evaluate the incidence of this mutation in these patients. It was noted that the incidence of the JAK2 V617F mutation in this study was lower in RARS-T and MDS groups [16-18]. This discrepancy might be caused by ethnic variation or by the population size being too small in previous reports as well as in this study. The incidence of the JAK2 V617F mutation in the AML cases in this study was 13.3% (2/15), which was within the range mentioned in previous reports.

The JAK2 V617F mutation is especially associated with increased platelet counts [19]. However, the exact mechanism underlying this increase is not known. From previous reports, megakaryopoiesis was enhanced when the progenitors are heterozygous for JAK2 V617F, whereas erythropoiesis is strongly stimulated when the progenitors are homozygous for the JAK2 V617F mutation [19]. In this study, patients with the JAK2 V617F mutation had an increased platelet count compared to those with wild-type JAK2, which was consistent with the results of a previous study [20]. These data suggest that the pathogenesis of thrombocytosis in patients with these 4 diseases might be similar to that in the case of essential thrombocytosis. Interestingly, 2 of 6 patients with the JAK2 V617F mutation showed a karyotype of del(20)(q11.2). Previous literature reported that 18.4% of primary myelofibrosis patients with the JAK2 V617F mutation harbored del(20)(q11.2) whereas 6.9% with the wild-type JAK2 harbored this karyotype, which is the 2nd most common karyotype after the normal karyotype [21, 22]. In this study, 1 MDS patient had both the JAK2 V617F mutation and harbored del(20)(q11.2). The del(20)(q11.2) karyotype was not found in RARS-T or AML patients. This result suggests that no correlation between the JAK2 V617F mutation and del(20)(q11.2) exists in cases of primary myelofibrosis, MDS, MDS/MPN-U, RARS-T, or AML. There were 2 more patients with del(20)(q11.2) among the remaining 37 (5.4%) without the JAK2 V617F mutation. This result suggests that del(20)(q11.2) might be associated with the JAK2 V617F mutation.

In conclusion, the JAK2 V617F mutation is associated with increased platelet count in MDS, MDS/MPN-U, RARS-T, and AML patients. Cytogenetic abnormalities of del(20)(q11.2) occurred in 1/3 of patients with the JAK2 V617F mutation, but further studies are required to confirm this association.

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