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RESEARCH ARTICLE

PREVALENCE AND SPECIFICITY OF MYELODYSPLASTIC SYNDROME WITH JAK2 MUTATION IN MOROCCO

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ABSTRACT

Background: The JAK2 V617F mutation has been noted in a majority of cases of polycythemia Vera, essential thrombocythemia, and primary myelofibrosis patients but occurs less frequently in myelodysplastic syndrome (MDS) about 5% of the cases; In Morocco, the Incidence of MDS will increase significantly in our country, MDS will be the disease of national public health. The aim of the current study was to determine prevalence of JAK2V617F mutation in Moroccan patients with MDS, and to compare characteristics of JAK2 mutation group to JAK2 wild type group. **Methods:** This prospective study included all patients who are newly diagnosed with MDS in hematology department 20 August hospital Casablanca from January 2014 to December 2015 period. The criteria of diagnostic were based on WHO classification, using blood, bone marrow analysis and karyotype. Group risk were defined according to the international prognostic scoring system (IPSS). Data obtained from JAK2 V617F mutation analysis and cytogenetic study as well as complete blood count and clinical data were analyzed. **Results:** we analyzed the JAK2 V617F mutational status in 49 MDS patients with sex ratio F/M at 1.3. The median age was 63 years old. The JAK2 V617F was present in 8% of cases. About mutated group, the median age was 74 years old. The platelet count was higher than 450000/mm³ in 2 cases, leukocyte count was also higher than 13000/mm³ in one case, hemoglobin count increased in 2 cases. Clinically one patient presented splenomegaly. According to WHO classification in this group mutated, one patient presented refractory anemia with excess blast (RAEB-1), one patient with MDS-U, and two cases of RCMD. About cytogenetic analysis the most common feature was normal karyotype and score intermediate 1 of IPSS risk score. **Conclusion:** The JAK2 V617F mutation is associated with older age and high platelet count with characteristics of MPN, the main cytologic and genetic features are the frequency of RCMD with normal Karyotype and intermediate prognosis, but further study are required to confirm this specificity.

INTRODUCTION

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by peripheral blood cytopenia, ineffective erythropoiesis and increased apoptosis with possibility to transform into leukemia (Ades, 2014). More than 50 recurrently mutated genes are involved in the pathogenesis of MDS, including genes that encode proteins involved in pre-mRNA splicing, epigenetic regulation and transcription (James, 2017; Adam, 2017). The Janus kinase 2 gene (JAK2) codes for a tyrosine kinase that is associated with the cytoplasmic portion of a variety of transmembrane cytokine and growth factor receptors important for signal transduction and phosphorylation in hematopoietic cell (Mc lornan, 2006).

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The JAK2 V617F mutation has been noted in a majority of cases of polycythemia Vera, essential thrombocythemia, and primary myelofibrosis patients but occurs less frequently in myelodysplastic syndrome (MDS) about 5% of the cases (Schmitt-graeff, 2008). In Morocco, the Incidence of MDS will increase significantly in our country, MDS will be the disease of national public health. Only few patients are reported in the national register, cytogenetic diagnosis remains incomplete and insufficient, so no molecular profile study has ever been performed. The aim of the current study was to determine the prevalence of JAK2V617F mutation in Moroccan patients with MDS, and to compare the characteristics of the JAK2 mutation group to JAK2 wild type group.

METHODS

This prospective study included all patients who are newly diagnosed with MDS in hematology department 20 August

hospital Casablanca from January 2014 to December 2015 period. The diagnostic criteria were based on WHO classification, using blood, bone marrow analysis and karyotype. We excluded all Patients with history of prior chemoradiotherapy and those with secondary MDS from this analysis. Collection of patient samples was approved by Written consent obtained from all patients. Blood and bone marrow smears were examined by using May-Grunewald-Giemsa and iron staining. The morphological analysis was reclassified by the cytologist in hematology laboratory department IBN Rochd in Casablanca, using WHO 2008 classification of myeloid neoplasm recommendation and adapted of the new 2016 classification. Cytogenetic analysis of bone marrow examination was done in a single private laboratory, at least 20 metaphases was analyzed and described according using G-banding and the international system for human cytogenetic Nomenclature recommendations. DNA was extracted from purifies granulocytes, and the JAK2 V617F mutation was detected systematically in blood by polymerase chain reaction specific allele with control on agarose gel. The genotyping by allele specific (AS-PCR) was performed using two forward primers and one common reverse primer (6). The sequences of the primers and probes are: Forward (specific):

5' –AGCATTTGGTT TTAAATTATG GAG TA TATT-3' Reverse: 5'-CTGAATAGTCCTAC AGTGTTTTC AGTT TCA-3' Forward (internal control): 5'-ATCTATAGTC ATGCTG AAAGTAG GAGAAAG-3' Group risk was defined according to the international prognostic scoring system (IPSS). Except for blood transfusion, the treatment wasn't standardized. The data were statistically analyzed using Excel Microsoft and statistical package for social Science version 20 (SPSS).

RESULTS

In this study, we analysed the JAK2 V617F mutation status in 49 MDS patients (29 females, 22 males) with a sex ratio F/M at 1.3. The median age was 63 years old (range 20-75).

Table 1. Clinical, biological and outcome features of patients

Mutation status	JAK2 wild type	JAK2 positive
Clinicals features:	n: 45	n:4
Median age	63	74
F/M	27/20	2/2
Anemia/ infection/bleeding	40/45	3/4
Tumoral syndrome	4/45	1/4
Biological features: (median)		
Hemoglobin (g/ 100 ml)	8	10.2
MCV Fl (median)	91	108
WBC (G/l)	3.5	13.5
Platelet (G/l)	116	560
Bone marrow blast (%)	8	1
WHO classification:		
RCMD / MDS-SLD	36	2
RARS / MDS-RS	1	0
RAEB-1/ MDS-EB	6	1
Unclassified/ MDS-U	2	1
Karyotype:		
Normal	30	3
Complex	3	0
Del 20 q	1	0
+8	2	0
Others	9	1
IPSS		
Low	5	0
Intermediate 1	33	4
Intermediate 2	4	0
High	3	0

MCV: mean corpuscular volume, WBC: white boold cell, WHO 2008 changed in 2016: world health organization MDS: myelodysplastic syndrome, RCMD/MDS-MLD: Refractory cytopenia with multilineage dysplasia /MDS with multilineage dysplasia RAEB/MDS-EB: Refractory anemia with excess blast/MDS with excess blasts, RARS: Refractory anemia with ring sideroblasts MDS-U: MDS unclassified , *Group risk according IPSS: International prognostic scoring system

The JAK2 V617F was present in 4 cases with an overall frequency of 8%. The clinical, biological features and outcome of patient with or without JAK2V617F mutation are summarized in the following table 1. In Group JAK2 negative, the median age was 63 years old with 60% female, anemia was less than 7g/dl in 85%, neutopenia less than 1000 elt/mm³ in 45% of patients and platelet count also less than 100000 elt /mm³ in 43% of cases. Using WHO classification, the majority were in refractory cytopenia with multilineage dysplasia subgroup (RCMD) 80%. Cytogenetic analysis of this group showed normal karyotype in 66%, and 73% of patients are in intermediate 1 score of IPSS. About mutated group, the median age was 74 years old with 2 men and 2 women. The platelet count was higher than 450000 elt /mm³ in 2 cases, leukocytosis count was also higher than 13000 elt /mm³ in one case, hemoglobin count increased in 2 cases harboring the JAK2 V617F mutation; and clinically one patient presented splenomegaly. According to WHO classification in this group mutated, one patient presented refractory anemia with excess blast (RAEB-1), one patient with MDS-U, and two cases of RCMD. About cytogenetic analysis 3 patients had normal karyotype and all cases have an intermediate risk score.

DISCUSSION

The MDS had heterogeneous features such as clinical, biological, cytogenetic and molecular profiles. In fact, molecular basis of MDS is characterized by a number of abnormalities, and the somatic mutation JAK2 V617F has been identified in rare case. The higher prevalence of this mutation has been found in myeloproliferative diseases (polycythemia Vera, essential thrombocythemia, and myelofibrosis with myeloid metaplasia, MDS/MPD) (Levine, 2005; Steensma, 2005). JAK2 is involved in cellular growth factor signaling, and deregulation of JAK2 by chromosomal aberrations may contribute to leukemogenesis (Parganas, 1998) with increased bone marrow cellularity. The JAK2 mutation identifies a subset of MDS patients with proliferative bone marrow morphology and frequent thrombocytosis, leukocytosis and splenomegaly (Gonzalez-Medina *et al*., 2002; Onida, 2004). The objective of this study was to detect the JAK2 mutation in cases of MDS excluded the MDS/MPN. The finding of JAK2 V617F mutation outside of the classical MPDs is uncommon with reports of low incidence in chronic myelomonocytic leukemia, atypical chronic myeloid leukemia, hypereosinophilic syndrome and chronic neutrophilic leukemia (Steensma *et al*., 2005), but the incidence is higher in cases of RARS-T (refractory anemia with ringed sideroblast associated with marked thrombocytosis) (Szpurka *et al*., 2006; Remacha, 2006). In our country the incidence of MDS is increasing, but no data and studies about the molecular profile of MDS; this work helps to identify a profile of a subgroup of Moroccan patients with MDS and their clinical and biological characteristics. This profile would be beneficial in establishing the prognosis and therapies that specifically target the mutant JAK2. We analysed 49 patients with MDS other than RARS-T, and we found a JAK2 mutation in 4 cases (8%), which is very rare. In French series (Renzis de, 2013) the incidence was higher with 28% JAK2 positive of 132cases, in Korea (Jekarl, 2010) the incidence is 13.9% (6/43); but in larger series in USA (Bejar *et al*., 2011), about 439 MDS only 3% of patient had a JAK2V617F mutation. Compared to the Maghreb country (Mervat, 2015), our results are higher than similar series with 5% of mutation.

The JAK2 positive cases have an older age (75 years old median VS 63 years) than group JAK2 wild type. This feature has also been observed in some study (Renzis, 2013; Jekarl, 2010; Bejar, 2011; Mervat, 2015). The mechanism of JAK2 V617F mutation causes an increase of platelet counts (Skoda, 2009). In this present study patient with mutation had an increased platelet count compared to those wild type, with hyperleukocytosis and clinically with 25% of tumoral syndrome (splenomegaly). This profile is rather compatible with manifestation of myeloproliferative disease and overlap syndrome (Fermo, 2007; Swerdlow, 2008; Basquiera, 2009).

According to WHO classification guidelines, we have identified MDS subtypes using the results of blood and bone marrow test. The 2008 classification revised in 2016 end redefine a new subtype of MDS (23). Outside the group of RARS-T, In the two groups JAK2 negative or positive the most frequent subtype was RCMD, similar of literature in French and USA (Renzis de, 2013; Jekarl, 2010; Bejar, 2011). Previous literature (Jekarl, 2010; Bejar, 2011; Mervat, 2015; Skoda, 2009; Fermo, 2007; Swerdlow, 2008; Basquiera, 2009; Arber, 2016; Millecker, 20104) reported that del (20) (q11.2) might be associated with the JAK2 V617F mutation. About 18.4% of primary myelofibrosis patients with the JAK2 V617F mutation harbored del 20q, whereas 6.9% with the wild type JAK2 harbored this karyotype, which is the 2nd most common karyotype after the normal karyotype. However, in this study, only one case of del 20q affects group with JAK2 negative, and the most common cytogenetic group was the normal karyotype. A large series have found no prognostic value to JAK2V617F mutation, although 64% of the JAK2+ cases had lower risk IPSS in bejar and al, confirmed by the French series with 80% of cases (Renzis, 2013; Jekarl, 2010; Bejar *et al*., 2011). Our prognosis was slightly higher, classified in intermediate 1 risk. In our context and given the little interest of this mutation in MDS, a further study is required to confirm this association and the specific feature.

Conclusion

The JAK2 V617F mutation was presente in 8% of MDS in Morroco. The research of this mutation was important, not only for differential diagnosis but this study helped to determine a specific profil of MDS with mutation, it is associated, with similar characteristics of MPN with older age and high platelet count. The RCMD classification with normal Karyotype and intermediate prognosis was the common subtype.

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Conflict of interests: The authors declare no competing financial interests.

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