RESEARCH ARTICLE

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Detection of TET2 Mutation in Patients with De Novo Acute Myeloid Leukemia: A Mutation Analysis of 51 Iranian Patients

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Abstract

Acute myeloid leukemia (AML) is a heterogeneous clonal disease that is considered to originate from hematopoietic stem cells, which are characterized by impaired myelopoiesis and blast proliferation. TET oncogene family member 2 (TET2) mutations are frequent in myeloid malignancies and several studies have assessed the clinical importance of TET2 mutations. However, its frequency ratio has not yet been fully clarified. **Method:** Hence, our study was aimed to analyze TET2mut in patients with de-novo AML and their association with clinical, molecular characteristics and Nucleophosmin 1 (NPM1), Fms-like tyrosine kinase 3 (FLT3), CCAAT Enhancer Binding Protein Alpha (CEBPA) and Wilms' tumor protein (*WT1*) gene expression. Fifty-one Iranian patients were screened by polymerase chain reaction (PCR) and direct sequencing to evaluate TET2 mutations frequency. **Results:** Out of all patients, 10 mutations in 8 patients (15.6%) were detected and closely associated with higher age and higher hemoglobin levels (p-value <0.05). Although *FLT3, NPM1* and *CEBPA* gene expression did not show any significant correlation with TET2mut, cytogenetically normal acute myeloid leukemia (CN-AML) patients appear to bear TET2mut more frequently with lower platelet counts. Monocyte-lineages leukemia has seemed to be more linked with TET2mut in these patients. **Conclusion:** Our study suggests the frequency of TET2mut in our study (15.6%) is in line with previous studies and reveals the critical role of TET2 in myeloid transformation, especially in leukemia with monocytic subtypes.

Keywords: Acute myeloid leukemia- TET2 mutation- Epigenetic- hematological malignancies- direct sequencing

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Introduction

Previous studies have revealed acute myeloid leukemia (AML) as a clinical and biological highly heterogeneous clonal disease that considered originating from hematopoietic stem cells, which characterized by impaired myelopoiesis and blast proliferation (Estey and Döhner, 2006; Saultz and Garzon, 2016). Genome-wide studies using next generation sequencing (NGS), direct sequencing and high-resolution single nucleotide polymorphism (SNP) array have been provided major insight into the panel of the prognostic markers of AML(Liersch et al., 2014; Mat Yusoff et al., 2019). These markers, which encompass genetic mutations, constitute the adoption of risk stratification and therapeutic decision-making in the overall survival of AML patients(De Kouchkovsky and Abdul-Hay, 2016; Kansal, 2016). In the last few years, studies concerning the prevalence and clinical outcome of the TET oncogene family member 2 (TET2) in a variety of myeloid malignancies(Rush and Plass, 2002). Alteration of TET2 was associated with critical changes in regular demethylation of 5-methylcytosine (5mc) to 5-hydroxymethylcytosine (5hmc) in DNA (Ito et al., 2010; Nakajima and Kunimoto, 2014). Somatic mutation in TET2 has led to dysfunctional progress of Hematopoietic stem cells (HSCs) as a result of the identification of TET2 alteration in patients with hematological malignancies such as Myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN)(Delhommeau et al., 2009; Solary et al., 2014). TET2 mutations were subsequently found in 7-27% of AML patients, 15-32% in cytogenetically normal acute myeloid leukemia (CN-AML) patients and 14-55% in other hematologic malignancies (Metzeler et al., 2011; Gaidzik et al., 2012; Weissmann et al., 2012). These studies also revealed that the clinical impact of TET2 mutations is not easily determined as a marker in patients with AML. In this study, we aimed to evaluate the prevalence and prognosis impact of TET2 mutations in 51 de-novo AML patients.

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Materials and Methods

Patient samples

The study comprised of 51 patients with newly diagnosed de-novo AML selected according to 2016 World Health Organization (WHO) diagnostic criteria(Döhner et al., 2010). Diagnostic peripheral blood (PB) and bone marrow (BM) samples were enrolled from two hematology centers (Ghaem and Imam Reza Hospitals, Mashhad, Iran). The current study was carried out in the Molecular Pathology Research center at Ghaem Hospital of Mashhad University of medical sciences and between May 2017 and May 2019. Improper samples, AML patients who have been treated with chemotherapy or had recurrent leukemia and uncertain diagnosis were excluded from the study. Out of 51 AML patients, 29 patients' clinical follow-up were available.

Analyses of TET2 mutation

Bone marrow and peripheral blood samples were collected at our laboratory on ethylenediamine tetraacetic acid (EDTA). Amplified genomic DNA by polymerase chain reaction (PCR) was attained to analyze entire coding exons (exon 3 to 11) of TET2 by using commercial kits (Fermentase, St. Leon-Rot, Germany). Sanger sequencing was performed to screen TET2 mutations by using forward and reverse corresponding primers which described by Gelsi-Boyer et al., (2009) study(Gelsi-Boyer et al., 2009). With included 20ng of genomic DNA for each PCR reaction, samples sequenced by ABI 3130 sequencer (PE Biosystems, Foster City, CA, USA). Databases used for single nucleotide polymorphism (dbSNP [http://www.ncbi.nlm.nih.gov/snp/] and Ensembl Genome Browser [http://www.ensembl.org /index.html]) to distinguish between polymorphisms and pathologic mutations. A variety of leukemic related gene expression such as Nucleophosmin 1 (*NPM1*), Fms-like tyrosine kinase 3 (*FLT3*), CCAAT Enhancer Binding Protein Alpha (*CEBPA*) and Wilms' tumor protein (*WT1*) were evaluated by real-time PCR method. That is also to say, to perform karyotype analysis, G-binding assay was utilized.

Statistical Analyses

All statistical analyses were two-tailed and P value of less than 0.05 was set for the level of clinical significance using SPSS version 11.5 (IBM, Chicago, IL, USA). Continuous variables are shown as median and range and for comparison with TET2 mutation statue Mann-Whitney U or Kruskal-Wallis tests were used. The Kolmogorov–Smirnov test selected to determine data distribution (the equality of continuous). Categorical variables are reported as count and relative frequency (percentage) and comparison with the groups was calculated by exact Fisher's test.

Results

Distribution of TET2 mutations in AML patients

As shown in Table 1, with a median age of 33.8 (range 2-79), 56% of all patients were male (29) and clinical characteristics such as median WBC 49.8 103/μL (1-234), Plt 81.9 103/μL (11-385) and Hb 7.92 g/dL (3-13) have attained. In all, frequency of NPM1, FLT3-ITD, CEBPA and WT1 expression were detected in 12, 14, 16 and 4 patients, respectively. A screen of cytogenetics subsets revealed that Intermediate individuals encompass the most cases of study with 32 patients (62%) which included 22 CN-AML cases (43%). Myeloid related subset of FAB classification such as M1, M2 and M4 included the majority of study patients (64%).

Out of 51 AML patients, TET2 mutations were detected in 8 patients (15.6%) with 10 mutations in all coding exons (exons 3-11) which out of all mutations, 4 mutations in exon 11 and 3 mutations in exon 3 were mostly affected exons. In addition, two patients harbored deletion-nonsense and insertion-missense mutations. According to our analyses (Table 2), patients showed 5 missenses (50%), 2 nonsenses (23%) and 2 deletion

Table 1. Clinical, Cytogenetic and Molecular Characteristic in 51 Children and Adults with de novo Acute Myeloid Leukemia According to the Presence or Absence of TET2 Mutations

Variant	All (n=51)	TET2mut (n=8)	TET2wt (n=43)	p
Median age, y (range)	33.8 (2-79)	49.6 (22-79)	30 (2-76)	0.04
Age in groups (percentage)				0.52
<15	13	0	13 (30)	
16-56	21	5 (63)	16 (37)	
56-85	13	3 (37)	10 (23)	
Sex				0.48
Female	22	3 (30)	19 (46.3)	
Male	29	5 (70)	24 (53.6)	
Laboratory data (range)				
WBC, 10^3/μL	49.8 (1-234)	26.9 (2.1-77.9)	55.4 (1-234)	0.112
Hemoglobin, g/dL	7.92 (3-13)	9.47 (6.2-13)	7.54 (3-11.3)	0.02
Platelets, 10^3/μL	81.9 (11-385)	55.0 (13-169)	88.5 (11-385)	0.39
FAB Classification (percentage)				0.44
M0	2 (3.9)	0	2 (5)	
M1	10 (19.6)	3 (38)	7 (16)	
M2	11 (21.5)	0	11 (26)	
M3	8 (15.6)	1 (12)	7 (16)	
M4	12 (23.5)	2 (25)	10 (23)	
M5	6 (11.7)	2 (25)	4 (9)	
M6	2 (3.9)	0	2 (5)	
Cytogenetics (percentage)				0.44
Favorable	13 (2505)	1 (12.5)	12 (28)	
Intermediate	32 (62.7)	7 (87.5)	25 (58)	
Unfavorable	4 (7.8)	0	4 (9)	
Missing	2 (3.1)	0	2 (5)	
NPM1 (percentage)	12 (23.5)	0	12 (28)	0.09
FLT3-ITD (percentage)	14 (27.4)	1 (12)	13 (30)	0.25
CEBPA (percentage)	16 (31.3)	2 (38)	14 (32)	0.6
WT1 (percentage)	4 (7.8)	1 (12)	3 (7)	1

Table 2. TET2 Somatic Mutations Found in 8 Patients Revealing 10 Different Mutations from Deletion and Insertion to Missense and Nonsense

ID	Sequence location	Consequence	Nucleotide change	Type	Cytogenetics
4152	3	P985FS	91021_91022insC	Deletion	46, XX (20)
4424	3	N338FS	89081_89082insT	Deletion	46, XY (20)
4468	3	P410FS	89289_89290insT	Insertion	46, XX (20)
4513	6	A1216C	92581T>C	Missense	46, XY (20)
3718	7	C1271W	3812C>G	Missense	46, XY (20)
4648	9	R1359C	92716C>A	Missense	46, XX (20)
4424	11	Q1852X	5554C>T	Nonsense	46, XY (20)
4199	11	G1796E	5387G>A	Missense	46, XY (20)
4184	11	R1516	4546C>T	Nonsense	46, XX (20)
4368	11	H1904R	5711A>G	Missense	46, XX (20)

Table 3. Association of TET2 Mutations with Other Gene Aberrations in All 51 AML Patients

Gene expression	All	$TET2^{mut}$	$TET2^{wt}$	p
FLT3-ITD	14 (27.4)	1 (10)	13 (31.7)	0.25
CEBPA	16 (31.3)	3 (30)	13 (31.7)	0.9
WT1	4 (7.8)	1 (10)	3 (7.3)	0.9
NPM1	12 (23.5)	0	12 (29.2)	0.09

mutations (31%). The harbored mutations in patients were detected as following: P985FS, N388FS and P410FS (exon 3), A1261C (exon 6), C1271W (exon 7), R1359C (exon 9), I1852X, G1796E, R1516N and H1904R (exon11). We did not find any relation between type and localization of mutations with FAB, karyotype or other clinical parameters. The 134920 substitution was the most frequented alteration, which confirmed as polymorphism.

Association with variables and other molecular alteration

As stated above in Table 1, TET2 mutation was associated with higher age and higher hemoglobin compare to those with wild type (P=0.04 and P=0.02, respectively). As a notable finding, our results did not reveal any mutations in patients younger than 15 years old and also, TET2mut patients older than 56 years have shown lower WBC count rather than wild-type patients (P= 0.024). Patients with CN-AML and those with lower WBC tend to reveal TET2 mutation more likely, although did not find any significant relation (P>0.05). That is to say CN-AML patients carrying TET2 mutations have shown lower Plt count (P=0.042). Within the group of aberrant karyotype, patients with intermediate karyotype were more frequently TET2-mutated (7/8, 87.5%) compared with other chrosomal aberrations, although did not find any significant relation (P>0.05). Furthermore, FAB classification analysis indicated that monocytic leukemia (M4 and M5) were the most effected patients but did not demonstrate any notable association. Regarding to molecular expression of all 51 patients, FLT3-ITD, NPM1, CEBPA and WT1 expression were not different between two patient groups (Table3).

Discussion

TET2mut have been identified in a variety of myeloid malignancies and are one of the most frequently acquired genetic alterations associated with MDS (Chan and Majeti, 2013). However, little is known about the prevalence and prognostic impact of TET2mut in de novo AML patients and inconsistent results within recent studies remain the prevalence and clinical role of TET2mut uncertain. While Gaidzik et al., (2012) revealed TET2mut in 7.6% of de-novo AML patients, Metzeler et al. disclosed that TET2 mut were found in 23% of AML patients, and Wiesmann et al. showed the 27% frequency of TET2mut in diagnosed patients. Our results demonstrated the overall TET2mut incidence of 15.6% in our cohort study. All mutations were heterogeneous and the mutations located outside the two conserved regions were excluded from the study. Furthermore, in line with Gaidzik et al., (2012) study and in contrast with others, most found TET2mut are missense and frame-shift alterations. To clarify the discrepancies, the similarity in population with the Gaidzik et al. study and different from others, the younger age of enrolled patients of the present study (median age of 33.8) might have been the explanation of discrepancies (Metzeler et al., 2011; Gaidzik et al., 2012; Weissmann et al., 2012).

In consistency with other studies, we noted that patients harbored TET2mut have had older age and higher Hb levels and tended to have lower WBC counts. Since CN-AML patients appear to bear TET2mut more frequently, these patients are related with lower Plt counts and tended to show lower WBC counts. Our investigations indicated that monocyte-lineages leukemia has seemed to be more linked with TET2mut which has been assumed to be a result of TET2 knockdown leading to impaired myeloid differentiation(Moran-Crusio et al., 2011; Walter et al., 2011). Metzeler et al. report were the only study in line with our findings(Metzeler et al., 2011). Regarding our analyses, the TET2mut did not show any correlation with FLT3, NPM1 and CEBPA expression levels(Ishfaq et al., 2012; Sarojam et al., 2015; Alarbeed et al., 2021). Since the previous studies evaluated the molecular prognostic markers through the sequencing method, this discrepancy might be caused by the difference in method.

To sum up, this study sequenced all coding exons of

TET2 in 51 patients with de-novo AML, indicates that TET2mut found in 15.6% of AML patients and had a significant association with higher age and Hb levels. However, due to insufficient data, we were unable to assess the exact role of TET2mut in overall survival.

Author Contribution Statement

Arbab Jafari designed the experiment; Chehreghani and Arbab Jafari analyzed the data and performed the experiment; Chehreghani and Bagheri wrote the paper; Ayatollahi and Sadeghian provided importnant materials; Zafari performed statistical analyses.

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Ethics Approval

This project did not require ethics approval, since all the process were done on samples were taken from patients thorough their routine program.

Conflict of interest

All authors indicate no potential conflict of interest.

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