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Incidence and Prognostic Impact of WT-1 Gene Exon7 and 9 Mutations in Acute Promyelocytic Leukemia

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ABSTRACT

Background: Wilms' tumor gene 1 (WT1) gene mutation has been reported to be a prognostic factor in normal-cytogenetic acute myeloid leukemia (AML) patients. Higher rates of mutation in the WT1 gene have been reported in several tumors including normal-cytogenetic AML patients. Data regarding WT1 mutations in acute promyelocytic leukemia (APL) is very scarce. In this study, we evaluated the incidence and impact of WT1 mutation on the outcome of APL patients.

Materials and Methods: A total of 92 patients diagnosed with APL were studied in three distinct groups: early mortality, relapsed, and persistent complete remission. Genomic DNA of bone marrow samples of patients was analyzed. For quantification of expression levels of the WT1 gene, real-time quantitative PCR (rqPCR) was performed by a real-time PCR system. WT1 mutation and its impact on prognosis were considered the primary endpoint of the study. Statistical analysis was performed with STATA.

Results: WT1 mutation frequency was 6.25% in the early mortality group (1/16 patients), 13.16% in the relapse group (5/38 patients), and 7.89% in the persistent complete remission group (3/38 patients). 8 mutations were in exon 7 and one mutation in exon 9. WT1 mutation in the relapse group was associated with a trend toward worse disease-free survival (DFS) while overall survival (OS) was not affected by WT1 mutation in univariate analysis. Patients with no mutations in WT1 and FLT3/ITD had better overall survival and disease-free survival compared to patients with mutations in the WT1 gene or FLT3/ITD in the relapse group.

Conclusion: The frequency of WT1 gene mutations does not differ significantly between patients with early mortality, relapse, and persistent complete remission. The presence of WT1 mutation is associated with higher relapse and lower survival rates in relapse group patients.

Keywords: WT1 mutation; Acute promyelocytic leukemia; Prognosis

INTRODUCTION

Wilms' tumor gene 1 (WT1) is a tumor suppressor gene located at 11p13.7 encoding a transcription factor[1]. This gene was originally found in WAGR patients and then its mutations in a proportion of Wilms' tumor children were reported[1,2]. The transcription factor encoded by WT1 is expressed in mesothelial cells, gonads and developing kidneys [3,4].

WT1 is also found in spleen and CD34+ progenitor cells but it is not detected in mature leukocytes[5,6]. Studies have shown that the WT1 gene is involved in various cellular and biological mechanisms such as cell apoptosis, differentiation, and proliferation [7,8]. Thus, it is theoretically expected that higher WT1 gene expression is observed in malignant conditions. Present evidence supports this idea with reports of overexpression of WT1 in several tumors such as pediatric tumors, mesothelioma, ovarian cancer, and hematopoietic cancers such as AML [4,9].

Studies have demonstrated altered levels of WT1 expression in the majority of AML patients [10-12]. It is also reported that the higher levels of WT1 in AML patients were found associated with poorer overall survival, higher relapse rates, and unresponsiveness to therapy [13]. Another study has also indicated that in treated cases of AML with detectable levels of WT1 transcripts, a higher rate of relapse is expected[12]. In addition to AML, overexpression of WT1 in CML and MDS is also well established. WT1 expression has been shown to correlate with higher counts of blast cells and elevated risk of myelodysplastic syndrome (MDS) conversion into AML (14). In addition to higher expression levels, mutations in WT1 exons 7 and 9 coding regions have been frequently documented in AML patients. 6-15% of AML patients harbor mutations of WT1 gene[3,15]. Several studies have concluded that these mutations worsen overall survival and relapse-free survival in AML patients. In contrast, studies exist which deny the association between the presence of these WT1 mutations and lower survival rates. Conflicting data are present in the literature regarding the impact of WT1 mutations on the prognosis of AML patients.

Acute promyelocytic leukemia (APL) as a distinct form of AML is currently a disease with a good prognosis but death in the early phases of disease or fatal relapses increase the mortality rate of this condition [13]. It is known that PML/RARA isoform and FLT3/ITD mutations are correlated with higher relapse rates[16] but the knowledge of WT1 mutations in APL is so little. The majority of studies on WT1 mutations have been performed in normal-cytogenetic AML patients. Thus, in this study, we aimed to evaluate WT1 gene mutations in patients with APL.

MATERIALS AND METHODS

This study has been designated as cross-sectional.

The patients were all selected from archives of Hematology, Oncology, and Stem Cell Transplantation Research Centre at Shariati Hospital, Tehran between 2001 to 2018. A total of 92 patients diagnosed with APL were studied in three distinct groups: early mortality, relapsed, and persistent complete remission. APL diagnosis was based on the detection of the PML-RARA fusion gene by PCR in all patients. All patients were treated with ATO or ATO+ ATRA as frontline therapy.

All the procedures performed in the study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments. (IR.TUMS.MEDICINE.REC.1395.1387) Informed consent was obtained from all individual participants included in the study.

Inclusion and Exclusion criteria

The criterion for inclusion in this study included the availability of a pre-treatment bone marrow or peripheral blood samples for gene mutation analysis. All patients signed the written informed consent forms for genetic analysis and the use of the laboratory results for scientific purposes.

Mutation and molecular analysis

Genomic DNA obtained from bone marrow samples of patients was analyzed using 3130 xl Genetic Analyzer (Applied Biosystems, USA) and Big Dye terminator V3.1 cycle sequencing method. Two primer pairs were used for polymerase chain amplification of WT1 exons 7 and 9. To confirm the found mutations, amplified and purified PCR products were sequenced. Molecular analysis of FLT3 internal tandem duplication was also conducted. To assess PML-RARA isoform type, total RNA from leukemic blasts was analyzed with the RT-PCR method. For quantification of *WT1 gene expression* levels, a real-time quantitative polymerase chain reaction assay was performed by a real-time PCR system with a WT1 Profile Quant Kit.

Study endpoints and statistical analysis

Overall survival (OS) was defined as the time from diagnosis to death. Disease-free survival (DFS) was also considered as the time from diagnosis to relapse, death, or last follow-up in complete remission (CR). CR was defined as normocellular bone marrow with <5% blasts, neutrophil count of 1×10^9/l, platelet count of 100×10^9/l, and normal morphology of other components. WT1 mutation and its impact on prognosis were considered as the primary endpoint of the study. The effects of FLT3/ITD and PML/RARA isoform on clinical outcomes were considered as secondary endpoints.

Statistical analysis was performed with STATA. Descriptive analysis was provided as frequency, percentages, mean and standard deviation. To compare patients based on various classifications, t-tests and chi-square tests were used for quantitative and qualitative variables. In cases without normal distribution, Mann-Whitney tests were used. Log-rank test for comparison of OS and DFS between groups was used and Kaplan-Meier estimation was used to produce survival curves. To determine prognostic factors of OS and DFS, Cox proportional hazard regression analysis was used for univariate and multivariate analysis. Statistically, significant definition was considered as $p < 0.05$.

RESULTS

Baseline characteristics

Ninety-two patients were analyzed in three groups: early mortality (EM) (16 patients, 17.39%), relapse (38 patients, 41.30%), and persistent complete remission (PCR) (38 patients, 41.30%). After stratification of risk groups, 33 patients (35.86%), 31 patients (33.69%), and 21 patients (22.82%) were assigned to low-risk, moderate-risk, and high-risk groups, respectively. Table 1 represents the details of the baseline characteristics of patients.

Frequency and characteristics of WT1 gene mutation

WT1 mutations were found in 9 patients (9.78%), including 8 cases of mutations in exon 7 and one mutation in exon 9. Mutation in exon 9 was a substitution mutation, while the form of mutations in exon 7 was frameshift change. These frameshift mutations were due to insertion, deletion, or a combination of these two types of alterations. A wild-type allele was present in all patients, which shows that all mutations were heterozygous.

WT1 mutation was present in one patient (6.25%), 5 patients (13.16%), and 3 patients (7.89%) in early mortality, relapse, and complete remission groups, respectively. No significant difference was detected between WT1 mutant and wild-type groups in terms of age, sex, FLT3/ITD, isoform, WBC count, hemoglobin concentration, and platelet count (Table 2).

FLT3/ITD mutation

FLT3/ITD mutation had been checked in 73 patients. 59 patients (80.82%) were negative for FLT3/ITD, while 14 patients (19.18%) were positive. FLT3/ITD mutation was positive in one patient (6.25%) in the early mortality group, 5 patients (13.16%) in the relapse group, and 8 patients (21.05%) in persistent complete remission. A comparison of parameters regarding the status of FLT3/ITD mutation is shown in Table 1.

Isoform

Isoform S was present in 9 (56.25%), 10 (26.32%), and 11 (28.95%) patients in the early mortality, relapse, and complete remission groups, respectively. These rates for isoform L were 7 (43.75%), 28 (73.68%) and 27 (71.05%). Isoform S was significantly higher in the early mortality group compared to other groups (p=0.02)

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Survival analysis

The median follow-up time for survival in patients with persistent complete remission was 62.58±11.79 months, while it was 120.04 ±12.2 months for patients in the relapse group. For all relapsed patients, survival analysis was performed with a median follow-up of 36 months. There was no significant difference in 3-year survival between patients with and without WT1 mutation. OS and DFS were relatively higher for wild-type WT1 patients compared to WT1 mutant ones in the relapse group but the difference was not statistically significant (p=0.09 and 0.06 for 3-year and 2-year DFS). Further analysis showed that DFS was

significantly lower in patients with FLT3/ITD+ compared to FLT3/ITD- patients in relapse group (36% vs 66%, p=0.001) (Table 2). Multivariate analysis showed that WT1-mutant patients had significantly lower DFS compared to wild-type patients. OS was not correlated with WT1 mutation status. FLT3/ITD+ was also correlated with lower OS and DFS in univariate and multivariate analysis (p<0.05).

Details of cox regression analysis are demonstrated in Tables 5 and 6. Sub analysis based on FLT3-ITD status revealed that patients with negative FLT3/ITD and wild-type WT1 had significantly better OS and DFS compared to those with positive FLT3/ITD or mutant WT1.

Table1. Patient's characteristics according to the WT1 mutation status

		WT1 mutation status		P
		Wild type (%)	**Mutant (%)**	
SEX	Female	44(95.65%)	2(4.35%)	0.07
	Male	39(84.78%)	7(15.22%)	
AGE	≤40	58(73.41%)	6(26.58%)	0.66
	>40	21(66.66%)	3(33.33%)	
FLT3/ITD	Positive	13(20%)	1(12.5%)	0.61
	Negative	52(80%)	7(87.5%)	
ISOFORM	Long	54(65.06%)	8(88.89%)	0.14
	Short	29(34.94%)	1(11.11%)	
Risk stratification	Low	30(90.91%)	3(9.09%)	0.39
	Intermediate	26(83.87%)	5(16.13%)	
	High	20(95.24%)	1(4.76%)	
WBC	≤10000	57(87.69%)	8(12.31%)	0.32
	>10000	20(95.24%)	1(4.76%)	
PLT	≥40000	32 (88.89%)	4(11.11%)	0.89
	<40000	44(89.80%)	5(10.20%)	
Fibrinogen	≤150	15(100%)	0(0%)	0.23
	>150	42(91.3%)	4(8.7%)	
Hemoglobin	<10	57(89.06%)	7(10.93%)	0.90
	≥10	8(100%)	0(0%)	

Table2. Univariate Cox proportional hazard regression analysis for OS and DFS

		OS		DFS	
		HR (CI %)	P	HR(CI%)	P
age		1.02(0.97-1)	0.36	1(0.97-1.04)	0.64
Sex	Male	Ref	0.95	Ref	0.36
	female	1.03(0.36-2.8)		0.68(0.3-1.58)	
FLT3/ITD	negative	Ref	0.36	Ref	**0.008**
	positive	1.8(0.51-6.37)		4.09(1.44-11.6)	
WT1	Wild	Ref	0.1	Ref	**0.07**
	mutant	2.5(0.82-7.61)		2.41(0.90-6.41)	
fibrinogen	≤150	Ref	0.12	Ref	0.75
	>150	5.17(0.65-41)		1.17(0.44-3.09)	
WBC	≤10000	Ref	0.4	Ref	0.74
	>10000	1.7(0.48-5.8)		1.2(0.41-3.4)	
HBG		1.2(0.96-1.4)	0.11	1/03(0.88-1.2)	0.6
Flt3/wt1	FLT3/ITD +orWT1+	Ref	0.05	Ref	**0.001**
	FLT3/ITD- & WT1-	0.37(0.13-1)		0.23(0.09-0.54)	

The abbreviations are as follows: **CI** confidence interval; **DFS** disease free survival; **OS** overall survival; **HBG**: hemoglobolin; **flt3/wt1**: FLT3/ITD +orWT1+ VS FLT3/ITD- & WT1-

DISCUSSION

Most studies assessed the prognostic value of WT1 mutation in non-m3 AML. The role of WT1 mutation in APL patients is unclear. We evaluated WT1 mutation in exon7 and exon 9 in addition to FLT3/ITD in APL patients.

The frequency of WT1 mutations was not significantly different between early mortality, relapse, and complete remission groups (6.25%, 13.16%, and 7.89%, respectively). Hou et al. [15] reported a rate of 6.8% for WT1 gene mutation in non-M3 AML. Gaidzik et al. [17] also reported that WT1 mutation was found in 12.6% of normal-cytogenetic AML patients. Krauth et al. [18] also reported a 5.5% rate for WT1 mutation in all AML patients and 10.97% for APL subjects. Gaur et al. [19] has reported that WT1 mutation is present in 4% of APL patients. Mutations on exon 7 were significantly more frequent than exon 9. This finding is consistent with previous reports.

The mean age of our patients was relatively lower compared to similar studies. We divided patients into two groups (over or under 40 years of age) and observed no significant difference in mutation frequency. Krauth et al. [18] reported a lower incidence of mutation in patients over 60 years of age. They suggested an inverse association of mutation with age. Becker et al.[20] also reported a higher frequency of mutation in normal-cytogenetic AML patients below 60 years of age in comparison to higher ages. We also observed higher mutation rates in ages below 60 years but the small sample size and younger population limited the scope of statistical analysis.

Isoform S in patients with early mortality was significantly more frequent compared to other groups. This finding is compatible with previous reports on the association of isoform S with remission and shorter survival.

Paschka et al.[21] revealed that FLT3/ITD was the second common abnormality in association with WT1 gene mutation. Our study showed no significant correlation between FLT3/ITD and WT1. Gaidzik et al.[17] also reported that the association of FLT3/ITD and WT1 was not statistically meaningful. Gaidzik et al. [17] reported that WT1 mutation has been associated with higher WBC counts. We did not detect any relationship between WBC counts and the presence of WT1 mutation.

Univariate analysis showed that patients with WT1 gene mutations had lower survival rates compared to patients without mutation OS: HR= (2.5(0.82-7.61), CI 95% P-value=0.1). Multivariate analysis ruled out the impact of WT1 gene mutation on overall survival. Univariate analysis revealed a 2.4-fold increase in the chance of relapse for patients with WT1 mutation (HR=2.41(0.90-6.41) CI 95%, p-value=0.07). Moreover, multivariate analysis showed a statistically significant association between WT1 mutation and relapse (HR=3.7 (1.28-10.5) CI 95%, p=0.01). One of the reasons for the lack of association between WT1 mutation and overall survival can be therapeutic interventions such as bone marrow transplantation, which is provided to patients in the second remission. Consistent with our findings, Krauth et al. [18] reported that mutation is associated with shorter DFS in normal-cytogenetic AML patients and does not correlate with overall survival. Yoon et al.[13] revealed that WT1 expression has been associated with a higher relapse rate and inferior disease-free survival in AML patients. Zidan et al.[16] reported that disease-free survival and overall survival were worse in patients with WT1 mutations compared to mutation-free patients. Paschka et al.[21] also reported that WT1 mutation has a significant impact on DFS and OS leading to lower survival and higher relapse rates. On the other hand, Gaidzik et al.[17] reported that the presence of WT1 mutation does not correlate with either OS or DFS. The authors attributed their results to the difference in the therapies used in comparison to other studies. Univariate analysis showed that the presence of mutation in at least one of WT1 or FLT3/ITD genes is correlated with higher mortality and relapse compared to patients without mutation in any of these genes. Although, multivariate analysis ruled out this association. Zidan et al. [16] has reported that patients with WT1 mutations show higher rates of resistance, relapse, and mortality compared to patients without WT1 mutations (p=0.041, 0.016, and 0.008, respectively). Gaidzik et al.[17] reported that patients with a mutation in both genes (WT1 and FLT3/ITD) has been linked to higher relapse, lower overall survival, and shorter remission period compared to patients with normal WT1 and FLT3/ITD.

CONCLUSION

The frequency of WT1 gene mutations does not differ significantly between patients with early mortality, relapse, and persistent complete remission. The presence of WT1 mutation may be associated with higher relapse and lower survival rates in relapse group patients. The absence of mutations in WT1 and FLT3/ITD genes may be correlated with lower relapse and higher survival rates compared to patients with mutations in at least one of the above genes.

Limitations

The limitation of our study is its low number of patients.Further studies, including a large number of patients are recommended to examine the association.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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