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RESEARCH ARTICLE

Prognostic Value of Fragile Histidine Triad Gene Expression in Egyptian patients with Acute Myeloid Leukemia

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Abstract

Background: AML is a relatively rare cancer, but is the most frequent form of acute leukemia. FHIT gene (Fragile Histidine Triad gene) is identified gene on chromosome 3p14.2 that has prognostic significance in AML. The aim of this work was to study the impact of FHIT gene expression on prognosis of AML in Egyptian patient.

Patients and methods: This study was conducted on 50 Egyptian patients with newly diagnosed AML who were subjected to full history taking, clinical examination and laboratory investigations including: complete blood count, LDH, bone marrow aspiration, Cytochemistry, immunophenotyping and assessment of FHIT Gene by real time PCR in bone marrow aspirate mononuclear cells.

Results: high gene expression was found in 26 cases (52%) and low expression in 24 cases (48%). High FHIT gene expression group includes 17 males (65.4%) and 9 females (34.6%) with mean age at presentation of 45.58 ±11.994 while low gene expression includes 12 males (50%) and 12 females (50%) with mean age at presentation of 39.17±12.967with no statistically significant differences between patients with high and low FHIT gene expression regarding age, sex and clinical presentations at time of diagnosis including pallor, splenomegaly, hepatomegaly and lymphadenopathy and laboratory investigations including WBCs and platelets counts, hemoglobin and LDH levels, and peripheral blood and bone marrow blast cell counts. There were statistically significant differences in disease outcome between high and low gene expression groups with higher rate of complete remission and Overall survival in high FHIT gene expression group compared with low FHIT gene expression group.

Conclusion and Recommendation: FHIT expression is an important prognostic factor in AML patients with normal Karyotyped and therefore we recommend its incorporation into novel risk-adapted therapeutic strategies to improve the currently disappointing cure rate of patients with AML.

INTRODUCTION

Acute myeloid leukemia (AML) is the most common acute leukemia affecting adults (4). AML comprises only 15% to 20% of these cases but accounts for a disproportionate 30% of deaths from acute leukemia. Approximately 20% of childhood leukemias are of myeloid origin and they represent a spectrum of hematopoietic malignancies (11). It is important to identify prognostic markers that patient's outcome more precisely, thereby allowing the development of

molecular risk-adapted treatment strategies that may improve the clinical outcome. By using the advanced technique quantitive real time PCR to detection any change in genes which play important role as independent prognostic factor in patients with cytogenetically normal AML (CN-AML).(9)

The FHIT gene which is tumor suppressor gene which located on chromosome 3p14.2 is deleted or inactivated in multiple human cancers. (2) Abnormal FHIT gene expression was found in a subset of patients with AML, acute lymphoblastic leukemia (ALL), and blast phase of chronic myeloid leukemia (CML), and (CLL) (12) .Additionally Homozygous deletions of the *FHIT* gene and absent or altered *FHIT* transcription are common in epithelial cancers such as lung (13), breast (10).

The prognostic significance of FHIT gene was shown in AML with association with significant lower refractoriness to induction treatment, higher rates of complete remission (CR), overall survival (OS) and disease free survival (DFS) for patients with high FHIT expression independent of other prognostic molecular markers with a gene expression signature consistent with less differentiated AML blasts

Results.

There were significant increase in FHIT CT, and significant decrease in its expression in AML when compared to control subjects. **(Table 1)** High FHIT gene expression was found in 26 cases (52%) and low expression in 24 cases (48%). High FHIT gene expression group (n=26) includes 17 males (65.4) and 9 females (34.6) with a mean age at presentation of 45.58 ±11.994. Fever /infection was found in 22 cases, Fatigue in 23 cases, Weight loss in 24 cases, Pallor in 24 cases, Bleeding tendency in 22 cases splenomegaly in 14 cases, hepatomegaly in 16 cases and lymphadenopathy in 12 cases. The low FHIT gene expression group (n=24) includes 12 males (50) and 12 females (50) with a mean age at presentation of 39.17±12.967. In this group Fever /infection was found in 23 cases, Fatigue in 23 cases ,Weight loss in 22 cases, Pallor in 19 cases, Bleeding tendency in 20 cases, splenomegaly in 15 cases, hepatomegaly in 16 cases and lymphadenopathy in 12 cases. There were no statistically significant differences between patients with high and low FHIT gene expression regarding age, sex and clinical presentations at the time of diagnosis. **(Table 2)**

There were no significant differences between high and low FHIT gene expression groups regarding WBCs and platelets counts, hemoglobin, and LDH levels, and peripheral blood and bone marrow blast cell counts. (The median WBCs count was 23.450 in the high FHIT gene group versus and 41.8 in the low FHIT gene group, with a p-value of 0.509. The median platelets count was 39.95 in the high FHIT gene expression group versus 33.00 in the low FHIT gene group, with a p-value of 0.600. The median hemoglobin level was 8.500 in the high FHIT gene group versus 7.25 in the low FHIT gene group, with a p-value of 0.361. The median peripheral blood blast cells in the high FHIT gene expression group was 40.50 versus 51.50 in the low FHIT gene expression group, with a p-value of 0.823. The median bone marrow blast cells in the high FHIT gene expression group was 67.00versus 75.50 in the low FHIT gene expression group with p-value of 0.325) The median LDH level was 838.61 in the high FHIT gene group versus 857.63 in the low FHIT gene expression group, with a p-value of 0.892. **(Table 2)**

There was anon significant association between high FHIT gene expression and FAB subtypes compared with low FHIT gene expression. In fact, of 26cases with high FHIT gene expression; 1 M0, 4 were M2,3 were M3, 8 were M4 , 6 were M5,3 were 6 and 1 M7 whereas of 24 patients with low FHIT gene expression; 1 M0,6 were M1, 3 were M2,5 were M3, 2 were M4 , 4 were M5,2 were 6 and 1 M7 **(Table 2)**.

There was a no statistically significant difference in disease outcome between high and low FHIT gene expression groups with lower rate of relapse, death and disease free survival while there was a statistically significant difference in disease outcome between high and low FHIT gene expression groups with higher rate of complete remission and Overall survival in high FHIT gene expression group compared with low FHIT gene expression group. Of 26 patients high FHIT gene expression; 21 achieved complete remission, 11 died and 2 suffered from relapse, while of 24 patients low FHIT gene expression; 10 achieved complete remission, 5 suffered from relapse and 15 died **(table 3)** with a mean Overall survival in the high FHIT gene expression group of 31.820 months compared with 22.089 months in low FHIT gene expression group, (p=0.049*) (**Table 4 and Figure 1**), and with a mean disease-free survival in the high FHIT gene expression group of 36.7months compared with 34.538months in low FHIT gene expression group. (p=0.136) **(Table 4 and Figure 2)**. Univariate and multivariate analysis for prediction of OS in AML patients was summarized in **(table 5).** Multivariate Logistic Regression Analysis of Biomarkers associated with risk of complete remission, Relapse and Refractory were summarized in **(tables 6, 7, 8)**. FHIT genes were considered independent protective factors against complete remission, relapse and refractory in AML.

Table (1): compare between FHIT gene expression in AML patients and healthy control

			Control (n=50)	AML (n=50)	P
FHIT	CT	Median (Range)	26.45 (26.4-30.7)	30.49 (21.6-39.7)	0.049*
		Mean± SD	27.714±2.02584	30.044±3.66884	
	Expression	Median (Range)	0.18049 (0.047-35.8)	0.0071393 ($2.50X10^{-5}$-0.637)	<0.001**
		Mean± SD	14.37±17.9133	0.056722±0.135777	

Table (2): Comparison between patients with high and low FHIT gene expression regarding clinical and laboratory data.

Parameters		High FHIT gene expression group (n=26)		Low FHIT gene expression group (n=24)		P- value
		N	%	N	%	
Sex	Male	17	65.4	12	50	0.271
	Female	9	34.6	12	50	
Age (years)	Mean ± SD	45.58±11.994		39.17±12.967		0.076
Fever /infection	Present	22	84.6	23	95.8	0.351
Fatigue	Present	23	88.5	23	95.8	0.611
Weight loss	Present	24	92.3	22	91.7	1
Pallor	Present	24	92.3	19	79.2	0.239
Bleeding tendency	Present	22	84.6	20	83.3	1
Splenomegaly	Present	12	46.2	15	62.5	0.222
Hepatomegaly	Present	14	53.8	16	66.7	0.586
Lymphadenopathy	Present	12	46.2	12	50.0	0. 0786
Total leucocytic count ($X10^9$/L)	Median(range)	23.450	1.4-213	41.8	3-176	0.509
Hemoglobin concentration (g/dL)	Median(range)	8.500	4.5-16.3	7.25	4.9-12.5	0.361
Platelets ($X10^9$/L)	Median(range)	39.95	5-249	33.00	2-143	0.600
Peripheral blasts (%)	Median(range)	40.50	16	51.50	17	0.823
Bone marrow blasts (%)	Median(range)	67.00	25-85	75.50	26-79	0.325
LDH (IU/L)	Median(range)	838.61	212-2342	857.63	192-3524	0.892
FAB classification						
M0	Present	1	3.8	1	4.2	
M1	Present	0	0	6	25.0	

M2	Present	4	15.4	3	12.5	0.130
M3	Present	3	11.5	5	20.8	
M4	Present	8	30.8	2	8.3	
M5	Present	6	23.1	4	16.7	
M6	Present	3	11.5	2	8.3	
M7	Present	1	3.8	1	4.2	

* Significant (p<0.05). SD=Standard deviation. TLC= Total leucocytic count. BM=bone marrow. LDH=lactate dehydrogenase.

Table (3): Outcome of studied patients in relation to FHIT gene expression

Expression	Groups	AML (n=50)				
		<median		≥median		*P*
FHIT	Total	24		26		
		N	%	N	%	
	CR	10	41.7	21	80.8	0.044*
	Refractory	4	16.7	1	3.8	0.340
	Relapse	5	20.8	2	7.7	0.417
	Total death	15	62.5	11	42.3	0.802

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	Expression	**Groups**	**< median**				**≥median**				***p***
			Cumulative Survival (%)	**Mean (months)**	**CI 95%**		**Cumulative Survival (%)**	**Mean (months)**	**CI 95%**		
FHIT	**AML**	**OS**	53.6	22.089	15.083	29.094	69	31.820	24.273	39.367	0.049*
		DFS	92.3	34.538	29.903	39.174	79.2	36.7	30.341	43.059	0.136

Table (4). Relation between survival times according to FHIT gene expression

Table (5).univariate and multivariate analysis for prediction of OS in AML patients.

		Univariate				Multivariate			
		P	HR	95% CI		*p*	HR	95% CI	
AML	Age (years)	0.709	1.006	0.973	1.041	0.931	1.002	0.967	1.037
	BM blasts (%)	0.574	1.095	0.976	1.093	0.459	0.993	0.976	1.011
	FHIT(above median versus below median)	0.021*	0.670	0.286	0.868	0.044*	0.504	0.201	0.964

Table (6). Multivariate Logistic Regression Analysis of Biomarkers associated with risk of complete remission in acute myeloid leukemia patients.

Diagnosis	Covariate	*P*	OR	95% CI	
AML	Age (years)	0.265	1.038	0.972	1.108
	BM blasts (%)	0.574	1.009	0.979	1.039
	FHIT (above median versus below median)	0.010*	2.5	0. 09	5.2

Table (7). Multivariate Logistic Regression Analysis of Biomarkers associated with risk of relapse in acute myeloid leukemia patients.

Diagnosis	Covariate	*P*	OR	95% CI	
AML	Age (years)	0.996	1.000	0.933	1.072
	BM blasts (%)	0.914	0.998	0.964	1.034
	FHIT (above median versus below median)	0.0419*	0.472	0.076	2.918

Table (8). Multivariate Logistic Regression Analysis of Biomarkers associated with risk of refractory in acute myeloid leukemia patients.

Diagnosis	Covariate	*P*	OR	95% CI	
AML	Age (years)	0.47	1.4	0.53	3.74
	BM blasts (%)	0.33	1.38	0.68	2.8
	FHIT (above median versus below median)	0.1	0.47	0.19	1.5

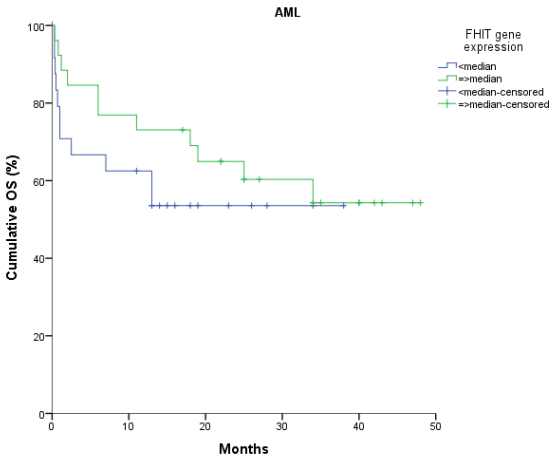

Figure (1): OS of AML patients according to FHIT gene expression

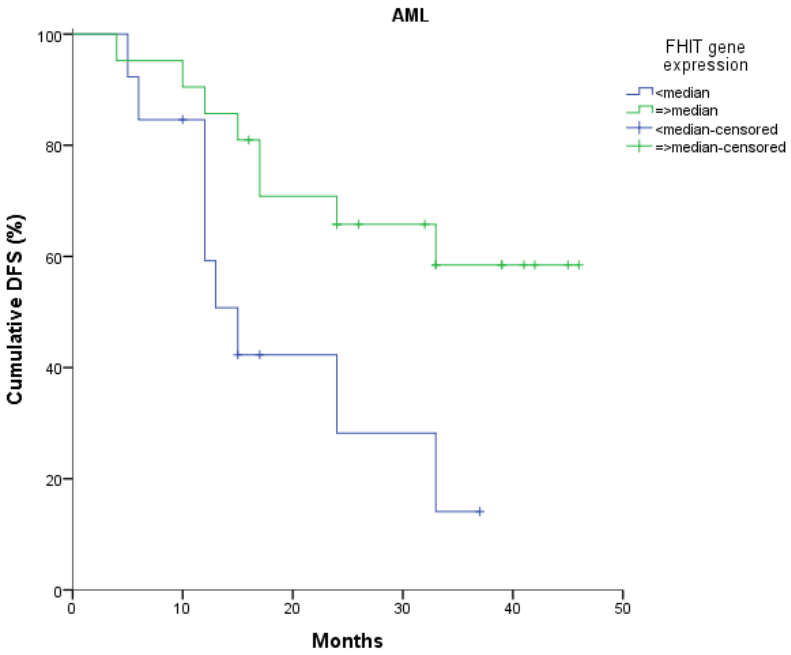

Figure (2): DFS of AML patients according to FHIT gene expression

Discussion.

Acute myeloid leukemia is a clonal malignant disease of hematopoietic tissue that is characterized by the proliferation of abnormal myeloblasts cells principally in marrow and impaired production of normal blood cells. The prognosis of AML varies dramatically and is strongly influenced by a number of factors, including age, performance status, and cytogenetic and/or molecular alterations (3).

In Multiple chromosomal and gene rearrangements have been identified in AML, such as MLL, PML/RARA, DEK/CAN, and AML1/ETO(8) Chromosomal rearrangements involving the MLL gene at band 11q23 are the most common genetic alteration encountered in infant acute myeloid leukemia. (6) The present study was designed to use Real-time PCR analysis to study the prognostic value of FHIT gene expression in Egyptian patients with AML.

In this study, low FHIT gene expression was found in 24 cases (48%). In previous studies, the reported frequency of FHIT alterations in hematopoietic disorders has varied; the expression of FHIT mRNA and/or protein was reported to be altered in 30–60% of AML. (7,14). Variation between the results of this study and the previous studies may be explained by different methods which used in detection ,different age and number of studied patients, different localities, different presentations of leukemia, different duration of studies and different duration of follow-up.

In the present study, there were no statistically significant differences between patients with high and low FHIT gene expression regarding age, sex and clinical presentation at time of diagnosis including pallor, fever, and weight lose, bleeding, hepatomegaly, splenomegaly, and lymphadenopathy. These results were in agreement with Jiang Lin et al. 2008 (5) who found no significant differences between high and low FHIT gene expression regarding clinical parameters of patients at the time of diagnosis.

In this work, there were no significant differences between high and low gene expression regarding WBCs and platelets counts, hemoglobin, and LDH levels, and blast cell counts in the peripheral blood and bone marrow. This is in agreement with Jiang Lin et al. 2008 (5) who found no significant differences between high and low FHIT gene expression regarding WBCs and platelets counts, hemoglobin, and LDH levels, blast cell counts, both in the peripheral blood and bone marrow.

In the current study, there was anon significant association between FHIT gene expression and certain FAB subtypes with predominant high FHIT gene expression in M0, M1 and M3, M7 and predominant low FHIT gene expression in M2, M4, M5, and M6. These data are in agreement with Wang L et al., 2003. (15)

In our study there were no statistically significant differences in disease outcome between high and low FHIT gene expression groups with lower rate of relapse and death and disease free survival while there were statistically significant differences in higher rate of complete remission and Overall survival in high FHIT gene expression group compared with low FHIT gene expression group. This is in agreement with Wang L et al., 2003 (15) who

stated that Kaplan-Meier plot of survival in patients with AML in relation to FHIT expression revealed that aberrance or loss of FHIT gene significantly correlated with a low clinical remission rate and poor overall survival. FHIT gene was considered independent protective factors against complete remission, relapse and refractory in AML this agree with Sugimolo. K et al., 1997

(12) Who suggested that loss of the normal FHIT function may be involved in the genesis of at least some human leukemias and that expression of aberrant FHIT transcripts is rather specific and frequent in leukemia samples.

Materials and Method.

This study approved by the ethical committee of Mansoura oncology institute and written consent was obtained from of all patients involved in this study. The study participants included 50 Egyptian patients with diagnosed AML being followed up under the Oncology Unit of the Mansoura oncology institute in the period from January 2013 to January 2015 including 29 males (58%) and 21 females (42%) with a mean age value of 42.50±12.759 years.

All patients were subjected to the following:

Three ml venous blood was collected under complete aseptic technique. They were delivered into 2 tubes: 1 ml blood into a tube containing EDTA for complete blood count and 2 ml blood into the plain tube for assessment of Lactate dehydrogenase levels. Two ml of bone marrow aspirate were drawn into a sterile tube containing EDTA for mononuclear cell separation for polymerase chain reaction (PCR).

Detection of FHIT Gene by quantitive real-time PCR.

To assess molecular responses. From each patient and healthy subject 3 ml of PB or BM samples were collected in sterile EDTA vacutainers, total RNA was extracted from PB or BM blood cells. FHIT mRNA expression was normalized to the simultaneously analyzed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The relative FHIT expression was determined using the comparative cycle threshold (CT) method. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and *FHIT* were co amplified in the same tube using 1 µL cDNA, 1× master mix (IQ Mix; BioRad, Munich, Germany), GAPDH probe (VIC-5-CAAGCTTCCGTTCTCAGCC-3-TMRA) with GAPDH forward (5'-GAA GGT GAA GGTC GGAGTC-3') and reverse (5'-GAAGATGGTGATGGGATTTC-3') primers, and *F*HITprobe (5'-(FAM)-TGA TGA AGT GGC CGA TTT GTT- (TAMRA)-3') with FHIT forward (5'-TGTCGTTCAGATTTGGCCAAC-3') and reverse (5' - TCATAGATGCTGTCAT TCCTGT -3') primers. Reactions were performed using real-time PCR 7000 sequence detection system (Applied Biosystems, Foster City, USA). Positive and negative controls were included in all assays.FHIT and internal control transcript levels were quantified using real-time PCR analysis (TaqMan) on an ABI prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Specific PCR products were amplified and detected using dual-fluorescent non-extendable probes labeled with 6-carboxyfluorescein (FAM), reporter and 6-carboxytetramethylrhodamine (TAMRA), quencher at 5`-end and 3`-end, respectively. The relative mRNA expression of FHIT transcript was calculated using the comparative cycle threshold (Ct) method.

Statistical Analysis. The patient's data were collected and statistically analyzed using SPSS software statistical computer package version 22. All Data were expressed as in terms of mean values ± SD, median (range) and number and percent. The difference between two means was statistically analyzed using the student (t) test. The log-rank test was used to assess survival. Significance was adopted at $p < 0.05$.

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