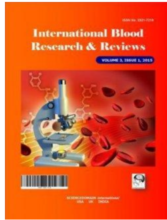

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Impact of DNMT3A Gene Mutation on Response of Acute Myeloid Leukemia Patients to Induction Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Author NR supervised the research work and approved the final manuscript. Author HF designed the study and wrote the protocol. Author DGS performed the investigations and the statistical analysis. Author NK managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the frequency and prognostic impact of DNA methyltransferase 3A (DNMT3A) gene mutation on response to induction therapy in newly diagnosed acute myeloid leukemia patients.
Study Design: Cross-sectional descriptive study.
Place and Duration: Hematology units of Suez Canal and Ain Shams schools of Medicine, Egypt. Between September 2016 and July 2017.
Methodology: The study enrolled forty patients (male: female ratio was 1; mean age was 52.4 ± 19.4 years) with newly diagnosed de novo AML before starting induction therapy. DNMT3A mutations were detected using dye terminator sequencing technique for the second part of DNMT3A, encompassing the PHD and methyltransferase domains and representing exons 11 till the last exon 23. Hematological, cytogenetic studies and DNMT3A mutation results were compared to the patients' hematological response to induction therapy.

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Results: Fourteen patients (35%) of the study participants had DNMT3A mutations while 65% had the wild type. Approximately 49.5% of mutations occurred in exon 23, the most common mutations were (R882C and R882H mutations; 28.5% and 21%, respectively). Out of 14 patients with DNMT3A mutation, 9 patients had incomplete remission and only 5 achieved complete remission with no statistically significant association. Odds ratio of the response to induction therapy according to DNMT3A mutation status was 1.32 times higher to show incomplete remission than in wild-DNMT3A patients.

Conclusion: DNMT3A mutation has high prevalence in AML Egyptian patients with non-statistically significant difference between mutated DNMT3A and wild type when related to the impact on remission rates after induction therapy.

Keywords: Acute myeloid leukemia; therapy; DNMT3A mutation; prognosis.

1. INTRODUCTION

Acute myeloid leukemia (AML) is a hematopoietic stem cell malignancy featured by enormous molecular heterogeneity, and epigenetic modifications of genes leading to increased proliferation and decreased differentiation of hematopoietic progenitor cells with the expansion of undifferentiated myeloid progenitors in the bone marrow and impaired hematopoiesis and bone marrow failure. AML is characterized by its great variability in clinical course and different prognosis and responses to therapy [1].

For the last two decades, genomic aberrations were known for their important role in the pathogenesis of AML, and cytogenetic aberrations have become well established diagnostic and prognostic markers. Moreover, microarray and next-generation sequencing technologies have increased our knowledge of the molecular heterogeneity of AML and enabled the identification of various mutations and distinct molecular subgroups in the context of recent disease classification and prognostic stratification [2-4]. Identification of novel risk-assessment biomarkers had a great impact on improving clinical care, and launching novel targeted therapeutic approaches [5]. Furthermore, the substantial heterogeneity in gene expression and DNA methylation profiles in AML were reflected in transcriptional and epigenomic studies [3].

Molecular markers in AML are crucial to patient characterization and improving risk stratification especially in cytogenetically normal AML patients (CN-AML) [6]. For instance, mutations in Nucleophosmin 1 (*NPM1*) and CCAAT/enhancer-binding protein alpha (*CEBPA*) are associated with a favourable outcome and lack of a transplant benefit [7], while, FMS-like tyrosine kinase 3 (*FLT3*) mutations are associated with adverse outcome [6].

Mutations in the DNA methyltransferase 3A (DNMT3A) gene in patients with AML have been identified [8]. DNMT3A mutation is one of the most frequent somatic mutations in AML. The probable mechanism by which DNMT3A mutations contribute to leukemogenesis is by altering DNA methylation and the attendant gene expression changes. DNMT3A mutations have been associated with a negative impact on overall survival (OS) [8]. In addition, the differences in global DNA methylation have been associated with differences in AML treatment outcome [9].

On the other hand, the time to achieve complete remission and the time to clearance of peripheral blast cells, after initial induction chemotherapy, have been reported as independent prognostic factors in patients with AML [10]. However, a more detailed analysis of the prognostic impact of DNMT3A mutations, in terms of relation to remission rate and relapse-free survival (RFS), in a uniformly treated AML patient cohort is missing [6]. The aim of this study was to assess the status and the relation of DNMT3A mutation to the response to induction therapy of a cohort of newly-diagnosed AML patients.

2. MATERIALS AND METHODS

2.1 Subjects

A total of forty patients with newly diagnosed AML were recruited from hematology units of Suez Canal and Ain Shams schools of Medicine, Egypt. Informed consent was obtained from every patient for laboratory studies and the study was approved by the Committee of Medical Ethics of Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Patients with antecedent hematologic diseases (e.g. chronic myeloproliferative disorder, MDS), therapy-related AML, solid tumors or bone marrow failure syndromes were excluded from the study.

Thirty-five patients were receiving induction chemotherapy protocol; in brief, one cycle of standard treatment (7+3) days protocol using Anthracyclin (usually idarubicin12 mg/m2/d on days 1-3 or Daunorubicin) and Cytarabine (seven days of Cytarabine 100 mg/m2/d). Five M3 cases received all-trans-retinoic acid (ATRA) for 40 - 45 days. The response to treatment was assessed after 28 days from induction therapy except for M3 after 45 days.

For assessment of the response to treatment, patients were distributed in terms of complete remission (CR) and incomplete remission. Patients with bone marrow blasts ≤5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; absolute neutrophilic count >1000/µL; platelets count >100.000/µL were considered to have complete remission. Patients with results other than the previous criteria were considered to have incomplete remission [11].

2.2 Methods

Complete blood counts (CBC) were performed by (ABX Micros 60, France). Peripheral blood and bone marrow (BM) smears were examined manually adopting the International Society for Laboratory Hematology (ISLH) screening criterion. CBC and BM aspiration with examination of Leishman stained smears were performed at day 28 after induction therapy to assess the extent of response to treatment. Immunophenotyping tests were done for the patients' BM samples at diagnosis by flow cytometry using (Epics XL flow cytometer, Coulter, USA). A panel of monoclonal antibodies including: T-cell lymphoid markers (CD2, CD3, CD5, CD7), B-cell lymphoid markers (CD10, CD19, CD20), myeloid markers (CD13, CD33), monocytic markers (CD14, CD11b) other markers (CD11c, CD15, CD61, cytoplasmic myeloperoxidase and glycophorin) as well as common progenitors' markers (CD34, HLA-DR) were purchased from Beckman coulter, Florida, USA. Samples were considered positive for a certain marker when expressed by ≥ 20% of cells, except for CD34, CD10 and cMPO where their expression by 10% of cells was sufficient to confer positivity.

Standard cytogenetic analyses were performed on metaphase chromosomes of harvested BM cells upon AML diagnosis before chemotherapy. The metaphase chromosomes were banded by the G-banding techniques and karyotype according to the International System for Human Cytogenetic Nomenclature. Twenty-nine patients were allocated to low-risk, intermediate-risk and high-risk cytogenetic risk groups according to ELN risk stratification [11]. The data were retrieved from the patients' files; cytogenetic studies were not available in eleven patients.

Genomic DNA isolation, PCR amplification and sequencing: Genomic DNA was extracted from mononuclear cells of AML patients. The purified genomic DNA was obtained using spin-column extraction method using QIAamp® DNA extraction kit (Qiagen, Germany). The DNA was extracted following the manufacturer's instructions. Nucleic acid samples were checked for concentration and quality using The Thermo Scientific NanoDrop™ 1000 Spectrophotometry and Agarose Gel Electrophoresis. Purified DNA was run as a single band on an agarose gel. The sequences of interest were amplified by the polymerase chain reaction (PCR) at the DNMT3A exons with the following primers: Forward primers: 5'ACGACAGCGATGAGAGTGAC3' and reverse primers: 5'CCCAATCACCAGATCGAATG3'.

PCR was performed in the presence of 25mM deoxynucleoside triphosphate, 20 pmol primers, 1mM MgCl2, DMSO, and 10 times buffer, Taq polymerase (Invitrogen, Life Technologies, Carlsbad, CA). Cycling conditions were as follows: 1 cycle for 5 minutes at 94°C, 35 cycles for 1 minute at 94°C, 1 minute at 56°C, 1 minute at 72°C, and 1 cycle for 10 minutes at 72°C [12].

The purified PCR products of approximately 1,400 base pairs were sequenced by using primers:

FWA,5_-ACGACAGCGATGAGAGTGAC-3, and FWB,5_GCTTTCTGGAGTGTGCGTAC-3.

Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA) and Sequencing products were resolved on an Applied model 3730XL automated DNA sequencing system (Applied BioSystems, USA). Sequencing was performed for the second part of DNMT3A, encompassing the PHD and methyltransferase domains representing exons 11 till the last exon 23.

2.3 Statistical Analysis

The statistical analysis of data was done by using SPSS program (SPSS, Inc., Chicago, IL), version 20.0. Quantitative data were presented as median, range, mean and standard deviation. Qualitative data were presented as frequency and percentage. The relation between DNMT3A mutations and various patient characteristics was determined by the chi-square test for the categorical variables. A univariate analysis was performed. For comparison between two groups; student *t*-test was used. Differences were considered significant when *P* values were lower than 0.05 at confidence interval 95.

3. RESULTS

The mean age of the studied patients was 52.4 ± 19.4 years. Table 1 represents the age and gender distribution and the hematological, cytogenetic risk stratification and response to induction therapy of forty Patients with AML according to DNMT3A mutational status. Patients distribution according to French-American-British (FAB) classification showed that the most frequent cases were M4 (37.5%) followed by M2 cases (22.5%), M3 (12.5%), M1 (10%), M5 (10%) and M0 (7.5%) with no cases with M6 or M7.

Applying the chi-square test showed that there was non-statistically significant association between gender or age with DNMT3A mutation status. Odds ratio showed that patients at age 60 and more are 3.54 times higher to show incomplete remission than those aged less than 60. Furthermore, there was a statistically significant association between DNMT3A mutation and total leucocytic count, blast cells in peripheral blood (%) and blast cells in BM (%) with *P*-values 0.001, 0.005 and 0.001 respectively.

Somatic mutations in DNMT3A were found in 14 patients (35%) with 49.5% of mutations occurring in the last exon 23. The most common mutations were a single nucleotide change in codon R882, from arginine to either cysteine or histidine (the R882C and R882H mutations were 4/14 (28.5%) and 3/14 (21%), respectively). Moreover, 28.48 % of mutations occurred at exon 19 (14.2% p.R736H, 7.14% p.R771Q, and 7.14% p.F752del). In addition, 14.28 % of mutations occurred at exon 15 (7.14% p.Q606X, and 7.14% p.R635W) and only one patient (7.14%) had mutation at exon 22 (p.S837XP) (Table 2, Fig. 1).

Patients were evaluated on day 28 (n=35) and day 45 for M3 cases (n=5) by peripheral and bone marrow smear examination for response to induction of therapy. Sixteen patients (40%) had clinical and laboratory evidence of CR after induction therapy, while 24 (60%) failed to respond to treatment. There were non-statistically significant differences between patients showing mutated DNMT3A and those with wild type. Whereas, 37.5% of patients with DNMT3A mutation had incomplete remission and 31.3% achieved complete remission. Odds ratio for the response to induction of therapy according to DNMT3A mutation status was 1.32 times higher to show incomplete remission than in wild cases. There was a statistically significant association between blast cells in peripheral blood (%) and blast cells in bone marrow (%) with response to induction of therapy with *p*-values 0.04 and 0.01 for blast cells (%) in peripheral blood and blast cells (%) in bone marrow, respectively (Table 3).

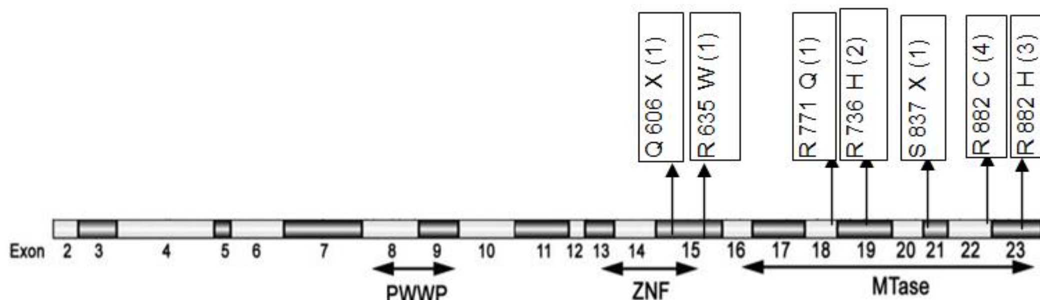

Fig. 1. Shows schematic summary of detected DNMT3A location and the frequency of gene mutations. DNMT3A gene and its domains: methyltransferase(MTase), zinc-finger (ZNF), and conserved proline-tryptophan-tryptophan-proline (PWWP). R, arginine; H, histidine

Table 1. Demographic, hematological and cytogenetic risk stratification of 40 patients with acute myeloid leukemia according to DNMT3A mutational status

Characteristic	*DNMT3A* mutated (n=14)		*DNMT3A* wild type (n=26)		*P* value
	No.	%	No.	%	
Age, years					.07
<60	5	35.7%	16	61.5%	
≥ 60	9	64.3%	10	38.5%	
Gender					.5
Female	8	57.2%	12	46.2%	
Male	6	42.9%	14	53.8%	
Hemoglobin, g/dL					
Mean ±SD	6.4±2.6		6.6±2.6		.8
Platelet count, x10^9/L					
Mean ±SD	50±25		36±27		.115
WBC, x10^9/L					
Mean ±SD	71.2±36.4		21.2±23.7		.001*
Peripheral blood blasts (%)					
Mean ±SD	38.6± 20.7		19.5± 6.2		.005*
Bone Marrow blasts (%)					
Mean ±SD	71.9±18.6		49.9±17.4		.001*
FAB category					
M0	1	7.1%	2	7.7%	
M1	0	0%	4	15.4%	
M2	6	42.9%	3	11.5%	
M3	0	0%	5	19.2%	
M4	5	35.7%	10	38.5%	
M5	2	14.3%	2	7.7%	
Cytogenetic risk stratification	n=10		n=19		
Favorable	1	10%	5	26.3%	
Intermediate	6	60%	10	52.6%	
Adverse	3	30%	4	21%	
Response to Induction therapy					0.7
Incomplete Remission (n=24)	9	37.5%	15	62.5%	
Complete Remission (n=16)	5	31.3%	11	68.8%	

**statistically significant P value*

Table 2. Mutation patterns found in 14 patients with DNMT3A mutations

FAB	Gene mutation	Exon	Protein	No.	%
M2, M5	c.2646G> A	23	p.R882H	3	21%
M2, M4, M5	c.2645C> T	23	p.R882C	4	28.5%
M4	c.1816C> T	15	p.Q606X	1	7.14%
M4	c.2510C> G	22	p.S837X	1	7.14%
M2	c.2255_2257del	19	p.F752del	1	7.14%
M2	c.2312G> A	19	p.R771Q	1	7.14%
M0, M2	c.2207G> A	19	p.R736H	2	14.2%
M4	c.1903C> T	15	p.R635W	1	7.14%

There was a statistically significant relation between age and response to induction therapy with *p*-value .02 and Odds ratio showed that patients at age 60 and more are 3.54 times higher to show incomplete remission than those aged less than 60.

Table 3. Hematological parameters in relation to the response to induction therapy

Response to induction therapy	Incomplete (n=14)	Complete (n=26)	P value
	Mean ±SD	Mean ± SD	
Hemoglobin, g/dL	6.7 ± 1.6	6.3 ± 2.5	.63
WBC, $x10^9/L$	44.6 ± 42.5	30.3 ± 26.4	.2
Platelet count, $x10^9/L$	41.3 ± 28.4	40.6 ± 26.5	.94
Peripheral blood blasts (%)	29.8 ± 18.9	20.8 ± 7.3	.04*
Bone Marrow blasts (%)	63.9 ± 20.03	48.1 ± 17.9	.01*

**statistically significant P value*

4. DISCUSSION

The issue addressed in the current cross-sectional study was investigating the impact of the mutational status of DNMT3A on the response to standard induction therapy in 35 AML patients and all-trans-retinoic acid in five M3 cases that might be a reflection of the overall survival in these patients. The demographic and hematological findings in the current study showed agreement with those of other studies performed on Egyptian AML patients [9,13,14].

The mutation frequencies in this study are comparable with those reported by other studies as in Ibrahem and coauthors [9]; who found that DNMT3A mutations occurred in 34 (28%) out of 120 patients, the most common mutation was R882H (n=23), followed by R882C (n=7), and R882S (n=4). Mutations at exon 23 occurred in all the 34 patients. The authors concluded that this type of mutation is frequent in Egyptians.

Tan and coworkers studied 157 patients, 33 patients were found to have DNMT3A mutations; the most common mutations were R882H and R882C [15]. Similarly, Amal and colleagues, studied 84 Tunisian patients and reported that DNMT3A mutations occurred in 33.3% of their AML patients [16].

This was not the case in Pezzi and colleagues study, which showed that DNMT3A mutation was found in 8% (6) of included patients. Of the 6 cases, the majority (83.3%) were located in exon 23 [17].

Induction therapy outcomes showed a non-statistically significant difference between patients with mutated DNMT3A and those harboring the wild type; 37.5% of patients with DNMT3A mutations had incomplete remission, while 31.3% achieved complete remission.

La Rochelle and coauthors (2011) reported that there was no measurable impact of DNMT3A mutation on the overall outcome for AML patients, but could be a predictive factor for response to Idarubicin, and thus, could have a direct influence on the way AML patients should be managed [18]. Ribeiro and co-authors studied the prevalence, the prognostic effect, and interaction with other molecular markers of DNMT3A mutations in 415 patients with AML and the results showed that DNMT3A mutation is not an independent prognostic value for complete remission in a multivariate analysis [12]. The same conclusion was reached by other studies [9,19]. Meanwhile, Thol and coworkers study reported that DNMT3A mutations predicted a lower CR rate in a multivariate analysis of patients with CN-AML [6].

These discrepancies may be related to differences in the size of patient populations analyzed, different ethnic background or using a scoring system integrating DNMT3A mutations with other molecular markers including NPM1, FLT3/ITD, CEBPA, RUNX and IDH2 which were not performed in the current study because of a financial issue.

In accordance to our observations, Marcucci and colleagues [20] reported higher white blood cell counts and percent of blast cells in bone marrow in mutated DNMT3A when compared to the wild type patients.

The current study shows a significant relation between age and response to induction therapy.

Some possible explanation for the relatively unfavourable clinical outcome in AML patients with DNMT3A mutations is the association of DNMT3A mutations with:

(1) Older age; Older patients have a higher incidence of multidrug resistance and an increased frequency of co-morbid medical conditions that affect the ability to tolerate intensive treatment (the current study shows statistically significant relation between age and response to induction therapy (*p-value*= .02); (2)

Statistically significant higher WBC; (3) Statistically significant higher blasts in DNMT3A mutated patients.

Moreover, the most common mutations were (R882C and R882H) which had certain biologic significance and induced protein change in the catalytic domain of DNMT3A protein which has a critical role in methyltransferase activity.

5. CONCLUSION

This study demonstrates that DNMT3A is frequently mutated in Egyptian AML patients. Such a mutation tends to be higher for patients presenting with higher white cell counts, blast counts and those above 60 years. Its prognostic impact in predicting induction response is yet to be more thoroughly studied. Our observations exhibit limited scope, primarily because the relatively small size. Larger cohort studies are warranted for setting a reliable risk stratification scoring system, integrating DNMT3A mutations with other prognostic factors including age, hematological parameters, cytogenetics and other molecular biomarkers for selecting AML patients who are candidates for intensive induction therapy.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that all procedures have been examined and approved by the ethics committee of Faculty of Medicine, Suez Canal University, Ismailia, Egypt. The research has therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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