RESEARCH ARTICLE

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Clinical Implication of *DNMT3A* and *TET2* Genes Mutations in Cytogenetically Normal Acute Myeloid Leukemia

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Abstract

Background: Refining risk stratification of cytogenetically normal AML (CN-AML) cases is important for decision making and tailoring of therapy. In this context genetic and epigenetic mutations was considered. Among these epigenetic regulators are *DNMT3A* & *TET2* genes. Therefore, the aim of this study was to determine the prevalence of *DNMT3A* and *TET2* genes mutations and their impact on the outcome of adult AML patients. **Subjects and methods:** The present study is cross sectional study which was conducted on 39 adult CN-AML patients at diagnosis. For all included patients sanger sequencing was done for *DNMT3A* exon 23 and *TET2* exon 3 genes. **Results:** *DNMT3A* mutations were detected in 8 of 39 patients (20.5%), and in 5 of 39 patients(12.8%) in TET gene. Two CN-AML patients had combined mutations in both genes. All of the mutations detected were missense and only one was frame shift. Mutated *TET2* or *DNMT3A* genes were significantly associated with failure of complete remission (CR) ($p < 0.001$), higher mortality rate, shorter OS (mean=16 versus 22.7 months) and shorter DFS (mean= 9.5 versus 21.4 months) when compared to non-mutated ones. **Conclusion:** Mutated *TET2* and *DNMT3A* detection define a subgroup of CN-AML patients with poor outcome.

Keywords: AML- *DNMT3A*- *TET2*- Epigenetic mutations- Outcome

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Introduction

Acute myeloid leukemia (AML) is an aggressive disease comprises a group of morphologically and genetically distinct malignancies characterized by an aberrant clonal proliferation of myeloid progenitor cells, hematopoietic stem cells or early myeloid precursors accumulate genetic and epigenetic alterations which lead to clonal expansion and eventually a block of myeloid differentiation (Uras et al., 2020).

Comprehensive cataloguing of the acute myeloid leukemia (AML) genome has demonstrated a high frequency of mutations of epigenetic factors that are linked to treatment resistance and poor patient outcome (Jones et al., 2020). Mutations of epigenetic modifiers founded in a huge proportion of AML patients, so represent important event for AML initiation, these epigenetic alterations are defined as changes in gene function that are inheritable through cell divisions but are not caused by DNA sequence changes.

Epigenetic changes contribute to AML pathogenesis, include mutations occur in genes involved in the regulation of DNA methylation (*DNMT3A*, *DNMT3B, TET, IDH1, IDH2*) or histone acetylation (*EZH2, MLL, ASXL1*), these epigenetic modifications are reversible, supported the rationale for the development of key clinical researches of novel epigenetic therapies in AML (Contieri et al., 2020). With the application of new molecular techniques, such as next-generation sequencing (NGS) technologies, certain genes mutations have been proved to be specifically related to the leukemia pathogenesis, such as mutated *DNMT3A* and *TET2* in AML patients which considered as pre-leukemia cells identification markers (Yu et al., 2020). *TET2* has a fundamental role in hematopoiesis, and enables the appropriate differentiation of hematopoietic stem cell HSC as involved in active demethylation of DNA via oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine, and 5-carboxylcytosine, mutations in *TET2* cause a significant reduction in 5hmC levels, increase hematopoietic stem cell (HSC) self-renewal and the expansion of myeloid lineage cells leading to development of AML (Das et al., 2021).

DNMT3A R882H have been found to be the most common mutation that encodes a dominant-negative protein which reduces methyltransferase activity by ~80% in cells with heterozygous mutations, causing a focal DNA hypomethylation phenotype (Ketkara et al., 2020). In recent years, although advances have been made in understanding the genomic landscape of AML and how some of these recurrent alterations cooperate to

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influence disease phenotype and prognosis, there is still a need to identify additional biomarkers to improve current risk stratification guidelines especially for those normal cytogenetic. Therefore, this study aimed to assess prognostic value of *DNMT3A* and *TET2* genes mutations in adult AML.

Materials and Methods

Subjects and Methods

The present study was carried out on 39 adult CN-AML patients. Mean age of patients was 48.2 years, they comprised of 18 males (46.2%) and 21 females (53.8%). The patients were selected from inpatient and outpatient's clinics of Mansoura University Oncology Center in the period between September 2016 to September 2018. All patients gave informed consent to participate in this study, and the IRB of Mansoura University approved the study protocol. Inclusion criteria included newly diagnosed CN-AML patients aged more than 16 years and less than 60 years. Exclusion criteria included patients aged less than 16 years, patients with 2ry AML leukemia and M3 FAB subtypes. The follow up period was for two years.

Treatment

Induction therapy: Seven days of standard-dose cytarabine (100-200 mg/m^2 /d) plus 3 days of Anthracycline (7 + 3) is the standard induction regimen in patients fits for intensive chemotherapy. Daunorubicin at a dose of 60-90 mg/m2 once daily or idarubicin at a dose of 12 mg/m^2 once daily for 3 days is the current Anthracycline of choice.

Post remission consolidation therapy: After the achievement of remission after induction, patients with an ELN favorable risk should proceed with chemotherapy consolidation only. However, patients with an intermediate or adverse risk should be consolidated with allogeneic stem cell transplantation.

Consolidation chemotherapy: Patients with favorable risk should be consolidated with chemotherapy using high dose cytarabine (HiDAC) 1.5-3 g/m^2 (every 12 hours in days 1, 3, and 5) for three cycles in patients age, 60 years or 1-1.5 g/m^2 for patients age 60 years and above.

Sampling

Venous blood samples 5ml were collected from each patient (2 ml of blood in plastic tube containing 50 µl of dipotassium EDTA solution for performing complete blood count and 3 ml in dry tube for biochemical analysis) and 5 ml bone marrow samples were collected from each patient before receiving any treatment were distributed between bone marrow smears for morphological examination, plastic tubes containing 50 µl of dipotassium EDTA solution for flowcytometry analysis, cytochemistry and molecular analysis and plastic tubes containing 50 µl sodium heparin for cytogenetic analysis.

Procedures

I- Routine laboratory investigations: Complete blood picture, biochemical analysis, Bone marrow examination, flowcytometry analysis, cytochemistry, molecular analysis and cytogenetic analysis.

Π-Specific laboratory investigation for identification of mutations in *DNMT3A*, *TET2* Genes:

1) Extraction of DNA: was done from EDTA bone marrow samples using Genejet genomic DNA extraction kit (Thermo Fisher Scientific) made in USA.

2) Amplification of DNA using PCR: was performed using the following primers for DNAMT3A exone 23 primers (BIOSEARCH, South McDowell Blvd, Petaluma, USA): Forward primer (5`- TCCTGCTGTGTGGTTAGACG -3`), Reverse primer (5`- ATGATGTCCAACCCTTTTCG -3`), TET2 exon 3 PCR1 primers (BIOSEARCH, South McDowell Blvd, Petaluma, USA): Forward primer (5`- TGAACTTCCCACATTAGCTGGT -3`), Reverse primer (5`- GAAACTGTAGCACCATTAGGCATT -3`), TET2 exon 3 PCR2: Forward primer (5`- CAAAAGGCTAATGGAGAAAGACGTA -3`), Reverse primer (5`- GCAGAAAAGGAATCCTTAGTGAACA -3`), TET2 exon 3 PCR3: Forward primer (5`- GCCAGTAAACTAGCTGCAATGCTAA -3`), Reverse primer (5`- TGCCTCATTACGTTTTAGATGGG -3`), TET2 exon 3 PCR4: Forward primer (5`- GACCAATGTCAGAACACCTCAA -3`), Reverse primer (5`- TTGATTTTGAATACTGATTTTCACCA -3`), TET2 exon 3 PCR5: Forward primer (5`- TTGCAACATAAGCCTCATAAACAG -3`), Reverse primer, (5`- ATTGGCCTGTGCATCTGACTAT -3`), TET2 exon 3 PCR6: Forward primer (5`- GCAACTTGCTCAGCAAAGGTACT -3`), Reverse primer (5`- TGCTGCCAGACTCAAGATTTAAAA -3`) Reactions were performed in a 25 µl volume containing 12.5 µL of Hot Star Taq Master Mix (Qiagen), 0.1µL of both forward and reverse primer, 1 µL of DNA, 11.3 µL of Nuclease free water. Amplification were done on 9700 thermal (Applied Biosystems, USA) with the following conditions for TET2: 95º C for 15 min, 35 cycles of 95º C for 30 s,35 cycles of 60º C for 30 s, 35 cycles of 72º C for 30 s followed by a final extension of 72º C for 10 min And with the following conditions for DNMT3A: 95º C for 5 min,40 cycles of 94º C for 30 s, 40 cycles of 55º C for 30 s, 40 cycles of 72º C for 1 min followed by a final extension of 72º C for 10 min then detection of PCR products was done on agarose gel 2%.

3. Sequencing: The amplified products of selected sample were programmed for the following:

-Purification of PCR product using Thermo Scientific Gene PCR Purification kit made in European Union.

-Cycle sequencing reaction using Big dye terminator V 3.1 cycle sequencing kit made in USA.

-Cycle sequencing purification using Centri-Sep spin column kit (Princeton separations, Adelphia, N.J.).

-Gene sequencing analysis using ABI 310 genetic analyzer (Applied Biosystems).

Statistical analysis

The statistical analysis of data was done using excel program (Microsoft Office 2013) and IBM SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups.

Quantitative data were presented by mean, SD, median and range. Comparisons between two groups were done using t-test or Mann Whitney (for non-parametric data). Logistic regression analyses was conducted for prediction of risk factors. (P is significant if <0.05 at confidence interval).

Results

Relation of genetic mutation with clinicopathological characters

The FAB subtypes of studied CN-AML groups are 10.3% M1, 33.3% M2, 41%M4, and 15.4% M5. The AML studied group was subcategorized based on cytogenetic findings into 30.8% is favorable, 41% is intermediate and 28.2% is unfavorable. Clinical characteristics of all investigated CN-AML patients are demonstrated in Table 1. *TET2* &*DNMT3A* mutations were significantly associated with older age (p= 0.002), but not with gender. The clinical findings as hepatomegaly, splenomegaly and lymphadenopathy was not significantly different between mutated and un-mutated CN-AML cases. *TET2* or *DNMT3A* mutations were significantly associated with higher total leukocytes count ,higher platelets count, higher peripheral blood and higher bone marrow blast cells count, lower hemoglobin. Likewise; *TET2* or *DNMT3A* mutations were frequently detected in M4 FAB subtype and low frequency in M2. On the other hand; no significant association was found with M1 and M5 (Table 1).

Frequency of genetic mutations among CN-AML

DNMT3A mutations were identified in 8 of 39 patients (20.5%), and *TET2* mutations in 5 of 39 patients (12.8%) in CN-AML patients. All types of the mutations detected were missense and only one was frame shift.

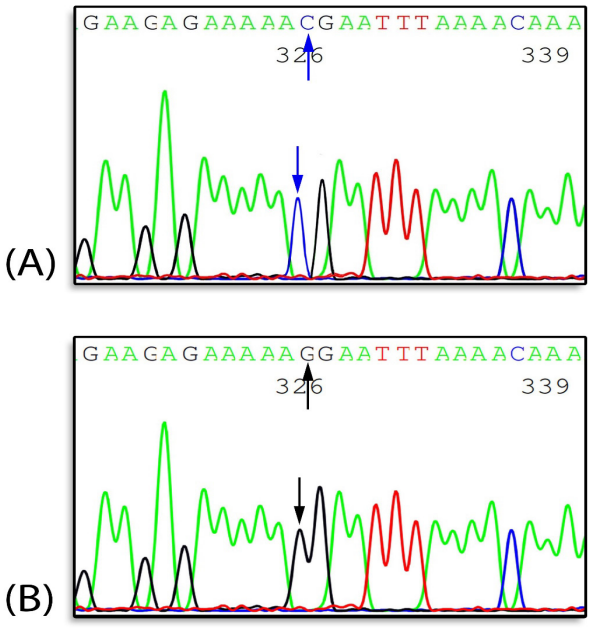

Figure 1. A, Missense Mutation C.2645G>C in exon 23 *DNMT3A* with Substitution of Guanine (G) with Cytosine (C). B, Non mutated *DNMT3A* sequence.

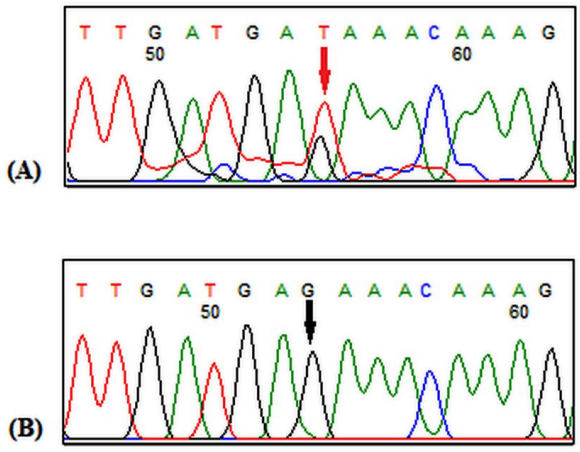

Figure 2. A, Missense Mutation C.2645G>C in exon 23 *DNMT3A* with Substitution of guanine (G) with cytosine (C). B, Non mutated *DNMT3A* sequence.

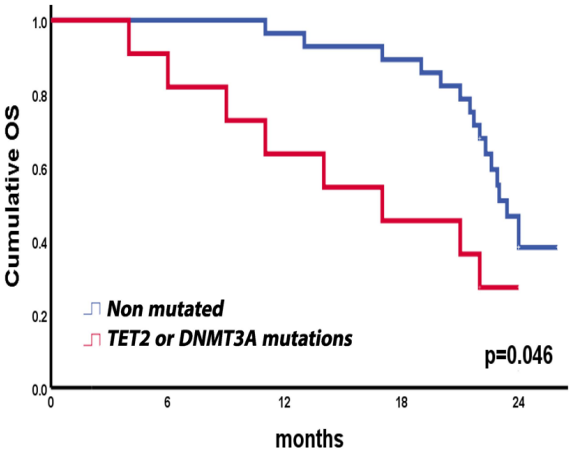

Figure 3. OS of CN-AML Cases with *TET*2 or *DNMT3A* Mutations Versus those with no Mutations. CN-AML patients with *TET2* or *DNMT3A* mutations had a significantly shorter OS when compared to non-mutated (P=0.046).

Two CN-AML cases harbored two genes (*DNMT3A* and *TET2*) mutations were shown in Table 2. Missense mutations of *DNMT3A* and *TET2* were depicted in Figure 1 and Figure 2.

CN-AML Survival Analysis

The mortality rate was more frequent in CN-AML patients harbored either *TET2* or *DNMT3A* mutations. Moreover; mutated CN-AML patients had a significantly shorter OS, DFS when compared to non-mutated (Figures 3,4 and Table 3). The impact of combined mutations on CN-AML OS and DFS did not significantly different when compared to CN-AML patients with single mutation (Figure 5 and Table 4). Cox regression analysis was conducted for prediction of OS in AML cases, using age, gender, marrow blasts, LDH, *DNMT3A* and *TET2* mutations as covariates. *DNMT3A* and *TET2* mutations were considered as independent risk predictors for shorter OS in AML cases in uni and multivariable analyses

Table 1. Characters of Studied AML Patients

		Non mutated N=28		TET2 or DNMT3A mutated N=11		p
Age (years)	mean±SD	46.6 ±12.7		60.6±10.1		0.002
Males	N, %	12	42.90%	6	54.50%	0.51
Females	N, %	16	57.10%	5	45.50%	
Hepatomegaly	N, %	22	78.60%	8	72.70%	0.693
Splenomegaly	N, %	20	71.40%	8	72.70%	0.935
Lymphadenopathy	N, %	5	17.90%	1	9.10%	0.655
Total leucocytic count ($X10^9$/L)	Median (range)	4 (1-125)		253 (5-500)		<0.001
Hemoglobin (g/dL)	Median (range)	9.2 (6.84-11.8)		7.3 (4.9-11.8)		0.04
Platelet count ($X10^9$/L)	Median (range)	32.5 (21-89)		83 (18-107)		0.001
PB blasts (%)	Median (range)	40 (19-53)		70 (53-80)		<0.001
Marrow blasts (%)	Median (range)	76 (65-90)		85 (75-90)		0.038
LDH (U/L)	Median (range)	748 (494-1767)		789 (494-1767)		0.38
FAB classification						
M1	N, %	4	14.30%	0	0%	0.309
M2	N, %	12	42.90%	1	9.10%	0.044
M4	N, %	8	28.60%	8	72.70%	0.027
M5	N, %	4	14.30%	2	18.20%	0.762
Induction of remission						
CR	N, %	25	89.30%	2	18.20%	<0.001
Failure of CR	N, %	3	10.70%	9	81.80%	
Patients Outcome						
Alive	N (%)	12 (42.9%)		3 (27.3%)		0.477
Died	N (%)	16 (57.1%)		8 (72.7%)		
OS	Cumulative OS (%)	38.2		27.3		0.046
	Mean OS (months)	22.7		16		

Table 2. Types of *DNMT3A* and *TET2* mutations in all studied AML cases

	Type	Nucleotide change	Amino acid change	Number
DNMT3A	missense	C.2645G>A	R882H	4
	missense	C.2644C>T	R882C	3
	missense	C.2645G>C	R882P	1
TET2	missense	G1933T	R534 I	1
	missense	G1285A	G429R	2
	missense	C817T	Q273X	1
	Frame shift	A3023ins	K1008X	1
Both DNMT3A & TET2	missense	C.2645G>A& G1933T	R882H& R534 I	2
		C.2645G>A& G1285A	R882H& G429R	

(Table 5).

Discussion

The prevalence of genetic mutations were identified in 8 out of 39 CN-AML patients (20.5%) regarding *DNMT3A* and in 5 out of 39 (12.8%), regarding *TET2* genes. Similar findings were reported by Wang et al., (2020) who reported that mutated *DNMT3A* was 19.3% and mutated *TET2* was 13.5% in AML patients. Also Zidan et al., (2018), Thol et al., (2011) and Ostronoff et al., (2013) reported that the incidence of *DNMT3A* exon 23 mutations was similar to our results (17.8%, 19% respectively). On the other hand Sasaki et al., (2020) found higher frequency of *DNMT3A* mutations (25%) and *TET2* mutations in 20%. This high figure could be attributed to large AML sample (n=421) and wide span of genes screening. On the other hand lower frequency was reported by Ponciano-Gómez et al., (2017) who found that mutated *DNMT3A* in 11.8%, and *TET2* in 2.7% in AML. This lower frequency could be explained on the basis of another race (Mexico). Regarding *DNMT3A* mutations the Brazilian study conducted by Ponciano-Gómez et al., (2017) found that incidence of *DNMT3A* mutations

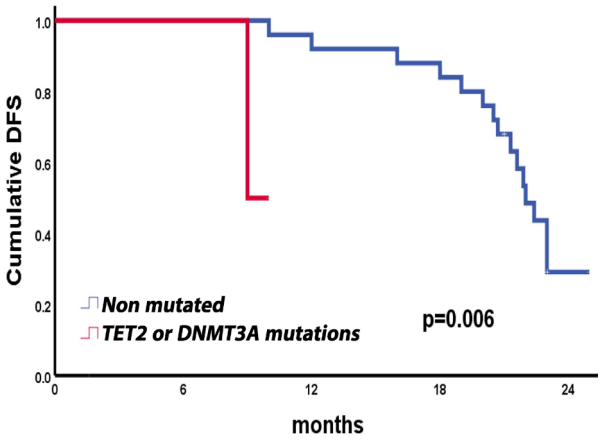

Figure 4. DFS of CN-AML Cases with *TET2* or *DNMT3A* Mutations Versus those with no Mutations. CN-AML patients with *TET2* or *DNMT3A* mutations had a significantly shorter DFS when compared to non-mutated ones (P=0.006).

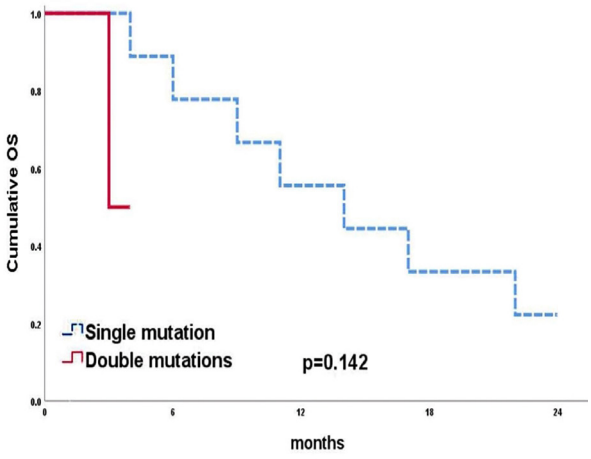

Figure 5. OS of AML Cases with *TET2* and *DNMT3A* Mutations. Single mutation, *TET2* or *DNMT3A* mutation; double mutations; *TET2* and *DNMT3A* mutations. No significant differences were found between AML patients had single and those had double mutations with mortality rate and OS among studied AML cases (p=0.142).

in CN-AML patients was 8% lower than the incidence reported in our study.

In the present study; the most common *DNMT3A* mutations were missense affecting Codon R882. While the most common *TET2* mutations which detected were missense mutations similar to that previously reported by Gaidzik et al., (2012) and Weissmann et al., (2012). A novel *TET2* mutation (G1933T/R534I) was detected in our study which not previously reported. Park et al., (2020) and Brunetti et al., (2017) founded that 65% of *DNMT3A* mutations affecting codon R882. Wang et al., (2019) study revealed that *TET2* mutations were distributed

Table 3. Impact of TET2 or DNMT3A Mutations on OS and DFS among Studied AML Cases

	Non mutated N=28	Mutated N=11	p
Alive N (%)	12 (42.9%)	3 (27.3%)	0.477
Died N (%)	16 (57.1%)	8 (72.7%)	
Mean OS (months)	22.7	16	0.046
DFS Mean OS (months)	N=25 (21.4)	N=2 (9.5)	0.006

Table 4. Impact of TET2, DNMT3A Single and Double Mutations on OS among Studied AML Cases

	Single mutation N=9	Double mutation N=2	p
Alive N (%)	2	1 (50%)	0.491
Died N (%)	7	1 (50%)	
Mean OS (months)	14.6	3.5	

OS, overall survival.

Table 5. Cox Regression Analysis for Prediction of OS

	Univariable		Multivariable	
	p	HR (95% CI)	P	HR (95% CI)
Age	0.108	1.060 (0.815-1.107)		
Gender	0.401	0.595 (0.177-1.998)		
BM blasts	0.177	1.051 (0.978-1.131)		
LDH	0.485	1.006 (0.995- 1.012)		
NC	0.118	1.028 (0.818- 3.191)		
DNMT	0.001	2.139 (1.602- 3.100)	0.004	2.218 (1.877-7.757)
TET	0.01	2.106 (1.486- 7.540)	0.02	2.409 (1.628- 9.248)

HR, hazard ratio; CI, confidence interval; NC, normal cytogenetics

throughout the whole gene with no specific hot spots. Missense mutations were the most common detected mutations among both genes in our study. Similar results was reported by Buscarlet et al.,(2017) who found that mutations in *DNMT3A* and *TET2* were spread over the entire coding sequence. Bussaglia et al ., (2017) reported that most type of *TET2* mutation detected was missense that affect *TET2* catalytic domain. Two AML cases (5.1%) harbored mutations in both genes (*DNMT3A* and *TET2*). These finding was in agreement with previous studies (Buscarlet et al., 2017, Damm et al., 2014, López-Moyado et al., 2019 and Moyado &Rao, 2020) who reported that *DNMT3A* and *TET2* mutations could be found either individually or in combined manner in myeloid and lymphoid malignancies.

Correlations studies between clinical and laboratory data of studied cases and *DNMT3A* or *TET2* mutations revealed that mutated *DNMT3A* and *TET2* were significantly associated with old age, high total leukocytes count, high platelets count, high peripheral blood blasts, high bone marrow blasts cell count when compared to non-mutated cases, while gender and clinical data did not differ significantly between both groups. Wang et al., (2019) reported that *DNMT3A* mutations were more likely to exist in older patient. Moreover, Veninga et al., (2020) reported *DNMT3A* mutations in AML patients are associated with higher platelets count as compared to those with non-mutated *DNMT3A*. This could be explained due to increased expression of inflammatory molecules that subsequently up regulate the thrombopoietin production by the liver. On the other hand, Asfour et al., (2020) detected no significant differences. Several reports (Panuzzo et al., 2020, Wang et al., 2019 and Kao et al., 2015) founded that *TET2* mutation was significantly associated with older age and higher white blood cell as compared to non-mutated *TET2* groups. These findings were in line with our results. In the contrast to our results Panuzzo et al., (2020) and Wang et al., (2019) observed lower platelets count in *TET2* mutated patients versus non mutated ones. Veninga et al., (2020) explained higher platelet count in *TET2* mutated patients as the TET gene product represses the transcription of inflammatory molecules, such as interleukin-6 and -8, which are known as pro-atherogenic mediators. So, somatic loss-of-function mutations in *TET2* are associated with an increased inflammation tendency that subsequently up regulate the thrombopoietin production by the liver resulting in higher platelet count.

In the present study M4 FAB subtype was the most frequent type associated with *DNMT3A*, and *TET2* mutations. These were in agreement with previous results (Pasca et al., 2020 and Wang et al., 2019) regarding *DNMT3A* mutations and Wang et al., (2019) regarding *TET2* mutations. In contrary to our results Asfour et al., (2020) found no association of *DNMT3A* mutations to any FAB subtype. Also, Chou et al., (2011) and Damm et al., (2014) didn't show any relationship between mutant *TET2* and FAB subtypes.

TET2 and *DNMT3A* mutations were significantly associated with normal cytogenetic. Similar association was addressed by previous reports (Pasca et al., 2020 and Park et al., 2020) regarding *DNMT3A* mutations and Panuzzo et al., (2017), Pasca et al.,(2020), Wang et al., (2019), Bussaglia et al., (2017) regarding *TET2* mutations. In our study, most of mutated *DNMT3A* and *TET2* AML cases expressed failure of CR, shorter OS and shorter DFS when compared to non-mutated CN-AML cases but the difference did not reach the level of significance. This was attributed to small number of mutated studied cases (n=11). These findings were in agreement with previous studies (Park et al., 2020, Wang et al., 2019, Bond et al., 2019 and Panuzzo et al., 2017) regarding *DNMT3A* mutations and similar findings by Wang et al., (2019) and Lin et al., (2017) regarding *TET2* mutations. On the other hand Panuzzo et al., (2017) and Zhang et al., (2020) found no significant associations of *TET2* mutations with shorter overall survival (OS).

DNMT3A and *TET2* mutations could affect the patients response to chemotherapy or increased AML patients mortality on the basis of the observation stated by Jaiswal and Libby(2020) who found that mutated bone marrow cells were associated with increased levels of circulating IL-8, an atherogenic chemokine, heart failure commonly complicates ischemic heart disease and is also strongly linked to ageing. Moreover, Jaiswal and Libby (2020) noticed altered immune cell function in the myocardium, and increased inflammation when the *DNMT3A* and *TET2* genes are mutated.

In conclusion, Mutated *TET2* and *DNMT3A* detection define subgroup of CN-AML patients with poor clinical outcome.

Conflict of Interest

The authors declare that there is no conflict of interest to declare

Author Contribution Statement

Salah Aref: Conception and study design and Manuscript revision; Nahed Sallam: Laboratory work; Interpretation and analysis of data; Sherin Abd Elaziz: Preparation of the manuscript and Revision for Important intellectual; Shimaa Al Ashwah: Clinical assessment of patients; Osama Salama: Supervision; Mohamed Ayed: Laboratory work; Interpretation or analysis of data.

Acknowledgments

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Ethical Statement

This study was approved by Mansoura Faculty of Medicine ethical committee and done according to declaration of Helsinki.

References

Asfour IA, Hegab HM, El-Salakawy W, et al (2020). Prognostic significance of *DNMT3a* gene expression and reactive nitrogen species in newly diagnosed Egyptian de novo adult acute myeloid leukemia patients. E*gypt J Med Hum Genet*, **21**, 34.

Bond J, Touzart A, Leprêtre S, et al (2019). *DNMT3A* mutation is associated with increased age and adverse outcome in adult T-cell acute lymphoblastic leukemia. *Haematologica*, **104**, 1617-25.

Brunetti L, Gundry MC, Goodell MA (2017). *DNMT3A* in Leukemia. *Cold Spring Harb Perspect Med*, **7**, a030320.

Buscarlet M, Provost S, Zada YF, et al (2017). *DNMT3A* and *TET2* dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood*, **130**, 753-62.

Bussaglia E, Anton R, Nomdedeu JF, et al (2019). *TET2* missense variants in human neoplasia. A proposal of structural and functional classification. *Mol Genet Genomic Med*, **7**, e772.

Chou W C, Chou S C, Liu C Y, et al (2011). *TET2* mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. *Blood*, **118**, 3803-10.

Contieri B, Duarte BKL, Lazarini M (2020). Updates on DNA methylation modifiers in acute myeloid leukemia. *Ann Hematol*, **99**, 693–701.

Damm F, Markus B, Thol F, et al (2014). *TET2* mutations in cytogenetically normal acute myeloid leukemia: Clinical Implications and Evolutionary Patterns. *Genes Chromosomes Cancer*, **53**.

Das AB, Díaz CCS, Vissers MCM (2021). Emerging epigenetic therapeutics for myeloid leukemia: modulating demethylase activity with ascorbate. *Haematologica*, **106**, 14-25.

Gaidzik V, Paschka P, Spa¨th D, et al (2012). *TET2* Mutations in Acute Myeloid Leukemia (AML): Results From a Comprehensive Genetic and Clinical Analysis of the AML Study Group. *J Clin Oncol*, **30**, 1350-57.

Jaiswal S, Libby P (2020). Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. *Nat Rev Cardiol*, **17**, 137-44.

Jones L, McCarthy P, Bond J (2020). Epigenetics of pediatric acute myeloid leukaemia. *Br J Haematol*, **188**, 63–76.

Kao HW, Liang D C, Kuo MC, et al (2015). High frequency of additional gene mutations in acute myeloid leukemia with MLL partial tandem duplication: *DNMT3A* mutation is associated with poor prognosis. *Oncotarget*, **6**, 33217-25.

Ketkara S, Verdonia AM, Amanda M, et al (2020). Remethylation of *Dnmt3a*−/− hematopoietic cells is associated with partial correction of gene dysregulation and reduced myeloid skewing. *PNA*, **117**, 3123–34.

López-Moyado IF, Tsagaratoua A, Yuitaa H, et al (2019). Paradoxical association of TET loss of function with genome-wide DNA hypomethylation. *PNAS*, **116**, 16933–42.

Moyado IFL, Rao A (2020). *DNMT3A* and *TET2* mutations reshape hematopoiesis in opposing ways. *Nat Genet*, **52**, 553–6.

Ostronoff F, Othus M, Ho P, et al (2013). Mutations in *DNMT3A* exon 23 independently predict poor outcome in older patients with acute myeloid leukemia: a SWOG report. *Leukemia*, **27**, 238–41.

Panuzzo C, Signorino E, Calabrese C, et al (2020). Landscape of Tumor Suppressor Mutations in Acute Myeloid Leukemia. *J Clin Med*, **9**, 802.

Park DJ, Kwon A, Cho BS, et al (2020). Characteristics of *DNMT3A* mutations in acute myeloid leukemia. B*lood Res*, **55**, 17-26.

Pasca S, Turcas C, Jurj A, et al (2020). The Influence of Methylating Mutations on Acute Myeloid Leukemia: Preliminary Analysis on 56 Patients. *Diagnostics*, **10**, 263.

Pezzi A, Moraes L, Valim V, et al (2012). *DNMT3A* mutations in patients with acute myeloid leukemia in South Brazil. *Adv Hematol*, **8**, 697691.

Ponciano-Gómez A, Martínez-Tovar A, Vela-Ojeda J, et al (2017). Mutations in *TET2* and *DNMT3A* genes are associated with changes in global and gene-specific methylation in acute myeloid leukemia. *Tumor Biol*, **2017**, 1–12.

Sasaki K, Kanagal-Shamanna R, Montalban-Bravo G, et al (2020). Impact of the variant allele frequency of ASXL1, *DNMT3A*, JAK2, *TET2*, TP53, and NPM1 on the outcomes of patients with newly diagnosed acute myeloid leukemia. *Cancer*, **126**, 765-74.

Thol F, Damm F, Lüdeking A, et al (2011). Incidence and prognostic influence of *DNMT3A* mutations in acute myeloid leukemia. *J Clin Oncol*, **29**, 2889-96.

Uras IZ, Sexl V, Kollmann K (2020). CDK6 Inhibition: A Novel Approach in AML Management. *Int J Mol Sci*, **21**, 2528.

Veninga A, Simone ID, Heemskerk J, et al (2020). Clonal hematopoietic mutations linked to platelet traits and the risk of thrombosis or bleeding. *Haematologica*, **105**, 2020-31.

Wang R, Gao X, Yu L (2019). The prognostic impact of tet oncogene family member 2 mutations in patients with acute myeloid leukemia: a systematic-review and meta-analysis. *BMC Cancer*, **19**, 389.

Wang RQ, Chen CJ, Jing Y, et al (2020). Characteristics and prognostic significance of genetic mutations in acute myeloid leukemia based on a targeted next-generation sequencing technique. *Cancer Med-Us*, **9**, 8457–67.

Weissmann S, Alpermann T, Grossmann V, et al (2012). Landscape of *TET2* mutations in acute myeloid leukemia. *Leukemia*, **26**, 934-42.

Yu J, Li Y, Zhang D, et al (2020). Clinical implications of recurrent gene mutations in acute myeloid leukemia. *Exp Hematol Oncol*, **9**, 4.

Zhang TJ, Zhang LC, Xu ZJ, et al (2020). Expression and prognosis analysis of DNMT family in acute myeloid leukemia. *AGING*, **12**, 14.

Zidan A, Elnaggar A, Zidan N, et al (2018). Clinical relevance of DNA methyltransferase 3a (*dnmt3a*) mutation in patients with acute myeloid leukemia. *Egypt J Haematol*, **43**, 193.

Zjablovskaja P, Florian MC (2020). Acute Myeloid Leukemia: Aging and Epigenetics. *Cancer*, **12**, 103.