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DNMT3A Gene Polymorphismin Iraqi Acute Myeloid Leukemia Patients and its Relation to Prognostic Factors and Response to Therapy

Alaa Saadoon Fadhil, Assistant prof. Dr. Maysem Mouyad Alwash and Assistant Prof Dr. Abeer Anwar Ahmed

Department of Pathology and Forensic Medicine,College of Medicine, Mustansiriyah University, Iraq.

ABSTRACT:

Acute myeloid leukemia (AML) is a complex and heterogeneous malignant disease of hematopoietic stem and progenitor cells characterized by the accumulation of immature blasts cells in the bone marrow, blood or other organs, that is related to environmental, genetic and epigenetic factors. Previous studies have shown that single nucleotide polymorphisms (SNP) of DNMT3A were correlated with the susceptibility of cancer, also suggested to play a crucial role in human cancer prognosis. The aim of the study is to assess the frequency of DNMT3A gene single nucleotide polymorphism (rs11695471) in Iraqi acute myeloid leukemia patients and it is association with disease development and response to treatment. The study included 80 persons, 40 AML patients and 40 healthy control age and sex matched. Routine investigations were collected which including symptoms and signs, physical examination, complete blood count, peripheral blood smears, bone marrow aspirates and immunophenotyping. RT (real time) polymerase chain reaction technique was used to detect DNMT3A gene polymorphism with Sa-cycler-96 well using genomic DNA isolated from peripheral blood and Taq Man SNP genotyping assay (rs11695471) for both patients and control. DNMT3A SNP rs11695471 was detected in all AML patients with 50% showing wild homozygous genotype and 50% showing the variant genotype, while 67.5% of control showing the wild homozygous genotype and 32.5% showed the variant genotype (P value=0.02). 77% of remission in AML cases are related to wild homozygous genotype (TT) of DNMT3A gene polymorphism, while 63% of non remission cases are related to the variant genotype(TA and AA). DNMT3A SNP rs11695471 is common among Iraqi AML patients and may force a high risk of AML development and influence response to treatment.

Key words: polymorphism, DNMT3A gene, acute myeloid leukemia, Iraq.

INTRODUCTION

Acute myeloid leukemia (AML) is a disorder characterized by a clonal proliferation derived from primitive haematopoietic stem cells or progenitor cells. Abnormal differentiation of myeloid cells results in a high level of immature malignant cells (myeloblast) accumulated in the bone marrow and peripheral blood and fewer differentiated red blood cells, platelets and white

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blood cells, results in a variety of systemic consequences including anemia, bleeding, and an increased risk of infection (Khwaja et al., 2016).AML has been considered a result of genetic alterations causing irreversible defects of the critical gene functions like proliferation, differentiation, apoptosis and gene transcription leading to leukemogenesis,The mutated genes are often graded in two classes : genes that confer a growth advantage including members of the signal transducer and activator of transcription (STAT), PI3K and RAS–MAPK pathways and genes which change the expression of key transcriptional targets in myelopoiesis such as PML, RUNX1 and MLL(Gutierrez and Romero-Oliva, 2013). Recently, the alterations in genes involving in the epigenetic regulation have emerged as the third class of gene mutations, these genes could affect the cellular differentiation and proliferation like DNMT3A gene (De Kouchkovsky and Abdul-Hay, 2016).

Gaining of DNMT3A, ten-eleven translocation2 (TET2) , and isocitrate dehydrogenase (IDH) 1 and 2 mutations in HSC can cause their clonal expansion resulting in a pre leukemia stem cell generation (Welch et al., 2012).DNA methyl transferase 3 alpha gene (109.63kb) is situated on chromosome 2p23.3 and contains 23 exons(Yang, Rau and Goodell, 2015)(Chen, Tsujimoto and Li, 2004).

DNMT3A is known as de novo methyltransferase, and are responsible for foundation of DNA methylation patterns via embryogenesis and build up genomic imprints through the germ cell development (Li and Zhang, 2014) (Zhang and Xu, 2017), through transferring the methyl groups from (S-adenosylmethionine to the 5-carbon) of cytosine, generating fifth-methylcytosine and homocysteine which perform the genomic methylation process(Moore, Le and Fan, 2013).Defective DNA methyltransferases produce imbalances in DNA and/or histone modification, so resulting in chromatin reconstruction, genomic instability and gene inactivation. Unequal with genomes in normal tissue, the tumor cells genomes mostly display global hypomethylation over, with localized hypermethylation in specific regions (Zhang and Xu, 2017b).

DNMT3A R882 mutation that usually acts as a poor prognostic marker in AML outcome (Kao et al., 2015), accounting for (18%–22%) of AML cases and in about (34%)of cytogenetically normal AML(Saultz and Garzon, 2016).

Previous researches elicited that DNMT3A gene polymorphisms were commonly associated with susceptibility of solid tumors, like gastric carcinoma (Wu et al., 2014), colorectal cancer (Zhao et al., 2012), hepatocellular carcinoma (Zhao et al., 2013), ovarian cancer (Mostowska et al., 2013), and breast cancer (Kullmann et al., 2013).

In addition, DNMT3A gene polymorphisms can lead to alterations in the methylation level of downstream genes, like LINE-1 and the imprinted gene (PEG3) (Tajuddin et al., 2013).

DNMT3A SNP (rs11695471) with others single nucleotide polymorphisms of this gene were genotyped in 344 diagnostic non- M3 chinease AML patients who received the standard

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combined chemotherapy with cytarabine and anthracyclines. DNMT3A SNP(rs11695471) was associated with decreased chemosensitivity , whereas SNP(rs11695471) was worse for overall survival. So, DNA methyl transferase 3 alpha polymorphisms may be a potential predictive signs for outcomes of AML patients, and that might improve the prognosis of AML(Yuan et al., 2016).

Although there is no report regarding the association between DNMT3A SNP(rs 11695471) in Iraqi population in general and AML patients in particular has bound us to embark on our research addressing the potential link between this variant and the risk and prognosis in Iraqi AML patients.

Aim of study

To evaluate the frequency of DNMT3A gene single nucleotide polymorphism (SNPrs11695471) in Iraqi AML patients and its association with disease development and response to treatment.

MATERIALS AND METHODS

Eighty subjects were included in this study: 40 AML cases were collected from the Medical City/ the National Center of Hematology, who were newly diagnosed as de novo AML andreceived the standard combined chemotherapy with cytarabine and anthracyclines with 40 who were age and sex matched as control group. Written informed consent was taken from every patient and approval of the Ethical committee was provided. Routine investigation were collected including clinical signs and symptoms, physical examination, complete blood count, peripheral blood smear, bone marrow aspirate and flow cytometry. A five ml of blood was collected in EDTA tubes. DNA extraction was done using WizPrep™ gDNAMiniKit (blood),(Cat.No:W71050-100, lot no. 3D0918-14). PCR (real time) was used to detect DNMT3A gene SNP by Sa-cycler 96 well using TaqMan® SNP Genotyping Assays[Applied Biosystems, USA] [Cat. No: 4351379]C-2096733-10, rs11695471, LOT:P190923-002 AD9. Patients with acute myeloid leukemia treated by “7+3” regimen and followed up for 21 days.

Statistical methods

Data were analyzed via the application of StatisticalPackage for Social Sciences (SPSS) version 23 “[SPSSInc., Chicago, IL]” and Microsoft excel program. Chi-square test and Fishers exact tests used for thecategorical variables.” One-way ANOVA “analysis wasused for the comparison of means for more than two groups.P value of “0.05 “or less was regarded as significant.

RESULTS

The mean age of AML cases was 42.5±15.74(Mean±SD)years and43.1 ± 16.44 (Mean±SD) years in controls “P value= 0.86”, male to female ratio 1.2:1. Pallor was the commonest presenting sign in AML patients with (82.5%) followed by fatigue (70%) and fever (62.5%).M2 was the most frequent subtype of AML (47.5%) followed by M5 (37.5%). Total WBC mean of

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the AML patients was 43±59.7 (mean±SD), mean of Hb for AML cases was 7.7±1.4 (mean±SD), 97.5% of them were anemic and platelet mean was 80.1±41.7 (mean±SD), 95% of patients were thrombocytopenic. The DNMT3A SNP rs11695471 wild genotype TT was more frequent among control (67.5%) compared to AML patients (50%), while the heterozygous TA and homozygous AA variant types were (27.5%, 22.5%) in AML cases respectively, and in control it was (30%, 2.5%) respectively (**p=0.02**), (Table1). DNMT3A polymorphism wasn't influenced significantly by age (p=0.7) and gender (0.3) (Tab. 2), AML (FAB) subtypes (P=0.5) (Tab.3) and bone marrow blast % (p=0.9) (Tab.4) .All patients with AML were treated with "7+3" regimen and followed up for 21 day after completion of treatment. Response to chemotherapy was classified for two groups remission and non- remission. DNMT3A SNP rs11695471 showed a significant statistical difference compared to response to treatment (**P value=0.05**) and to confirm these finding, we used the dominant genetic model (AT+AA) versus the wild type(TT) which showed a significant difference(**p=0.01**) . the homozygous wild type was showed the higher remission percentage (77%) while the variant genotypes (AT, AA) showed higher non remission percentage (33.4%) and (29.6%) respectively (Tab.5)

Table 1: Distribution of DNMT3A SNP (rs11695471) genotypes according to study groups

Variable	Study groups				P
	AML case		Control		
	No.	%	No.	%	
DNMT3A SNP (rs11695471) Polymorphism					0.02 *s
TT	20	50.0	27	67.5	
TA	11	27.5	12	30.0	
AA	9	22.5	1	2.5	

*S significant

Table 2: Distribution of DNMT3A SNP (rs11695471) genotypes according to age and gender in AML patients

Variable	DNMT3A SNP(rs11695471) Polymorphism						P
	TT		TA		AA		
	No.	%	No.	%	No.	%	
Age							0.7 * NS
<40 years	7	35.0	4	36.4	4	44.5	
40-64 years	8	40.0	6	54.5	5	55.5	

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≥65 years	5	25.0	1	9.1	0	0	0.3 * NS
Gender							
Male	13	65.0	4	36.4	5	55.5	
Female	7	35.0	7	63.6	4	44.5	

* NS not significant

Table 3: Distribution of DNMT3A SNP (rs11695471) genotypes according to AML subtypes

Variable	DNMT3A SNP(rs11695471) Polymorphism						P
	TT		TA		AA		
	No.	%	No.	%	No.	%	
AML subtypes							0.5* NS
M0	2	10.0	0	-	0	-	
M1	2	10.0	0	-	0	-	
M2	10	50.0	5	45.5	4	44.5	
M4	1	5.0	1	9.0	0	-	
M5	5	25.0	5	45.5	5	55.5	

*NS not significant

Table 4: Distribution of DNMT3A SNP (rs11695471) genotypes according to blast %

Variable	DNMT3ASNP(rs11695471) Polymorphism			P
	TT	TA	AA	
	Mean±SD	Mean±SD	Mean±SD	
BM blast (%)	63.8±17.6	63.1±22.6	61.5±17.6	0.9* NS

*NS not significant

Table 5: Distribution of DNMT3A SNP (rs11695471) Polymorphism according to treatment response

Variable	Response				P
	Remission		Non-remission		
	No.	%	No.	%	
DNMT3A SNP (rs11695471) Polymorphism					0.05*S
TT	10	77	10	37.0	
TA	2	15.4	9	33.4	

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AA	1	7.6	8	29.6	
DNMT3A genotypes (dominant model versus TT)					**0.01*S**
TA+AA	3	23.0	17	63.0	
TT	10	77.0	10	37.0	

*S significant

Discussion

The mean age of the AML patients included in this study was 42.5±15.74, which is similar to Iraqi study done byTawfiq et al (2019), in which the mean age was 42 years. Also, there was accordance with mean of age from others Iraqi and Egyptian studies with 45.51 and 44.5 years reported by Abdulateef,et al (2017) and Zidanet al (2018) respectively.

AML is slightly more common in men, in this study gender distribution showed slight male predominance that was corresponding with other studies from developed and developing countries presented by Maksimovic et al (2018),Sultan (2016),Muhsin and Al-Mudallal (2014), Silva-Junior et al (2019) andElasbali et al (2019).

Pallor and easy fatigability were the commonest signs and symptoms that found in 82.5% and 70% respectively, these findings agreed with Iraqi study reported by Alwanet al (2009)who found that pallor and fatigue was presented in 83% and 70% of AML patients in his study, our findings also close to Egyptian studyby Mahmoud *et al (* 2006) in which pallor was the commonest clinical presentation with 87% . Fever was found in 62.5% of the patients in this study and this agreed withIraqi study conducted by Al-Bayaa, Al-Rubaie and Al-Shammari(2020) with 63% . While others symptoms like hepatosplenomegaly, bleeding tendency and weight loss were presented with lesser extent and this was similar to others Iraqi study conducted byAlwan, (2009) , Arabic research by Abuhelwa et al(2017) and another study from Pakistan presented by Asif and Hassan(2013) .

The major portion of AML subtypes in our study was M2 subtype (47.5%), followed by M5 subtype (37.5%) and these results were similar to others Iraqi report conducted by Al-Bayaa, Al-Rubaie and Al-Shammari (2020)(50%,30%).

The mean of WBC was 43±59.7, while mean Hb value was 7.7±1.4 and platelet count with mean of 80.1±41.7 for AML cases, These findings were parallel to Iraqi study byJalal (2018), in which the mean of WBC count, Hb and platelet count were 47.1+71.7 x 10^9/L, 7.7+3.3 g/dl, 84.1+118.6 respectively.

The frequency of DNMT3A SNP rs11695471 showed a significant difference (**p=0.002**) between the study groups (Table 1). A higher wild homozygous type (TT) expression was found among control (67.5%) in comparison to patients (50%), heterozygous variant genotype (AT)was expressed slightly higher among control (30%) than patients (27.5%), while homozygous variant (AA)was more expressed in patients (22.5%) than control (2.5%),according to our knowledge,

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this study is the first research investigated the distribution of DNMT3A polymorphism SNP (rs11695471) in Iraqi healthy population and AML patients, no previous reports regarding the possible association between AML susceptibility and DNMT3A polymorphisms,Chinese study presented by Yuan et al at 2016,in which SNP(rs11695471) was one of a number of DNMT3A polymorphisms genotyped in AML patients, their study included the evaluation of DNMT3A polymorphism as predictive marker in AML outcome,they found three genotypes for SNP (rs11695471) in AML patients included the wild homozygous type TT (83%), heterozygous variant TA , and the homozygous variant genotype AAtogather with(17%).

Our results showed a high prevalence of the homozygous variant genotype AA in patients than the controls, which may be a relative risk factor in the development of AML disease, no previous studies regarding SNP (rs11695471) and AML risk, therefore our findings may be by chance and to confirm our suggestions, further studies with larger sample size is recommend priority in the future .

No a significant statistical difference regarding age (p=0.7), gender (p=0.3), AML subtypes (p=0.5), and B.M blast %(p=0.9) between patients expressing different DNMT3A genotypes which comes in agreement withYuan et al (2016).

Our results revealed a significant statistical difference with P value equal to (**0.05)** regarding the association between the genotypes and response to treatment after the first cycle of induction therapy with cytarabine and anthracyclines.We observed that wild homozygous genotype TT had higher prevalence of remission and chemosensitivity (77%) than TA and AA genotypes with (15.4%, 7.6%)respectively. Because the small size of cases and to confirm our results, therefore we used the dominant or combined genetic model (TA+AA) versus the wild genotype (TT) to assess the association between polymorphic genotypes and response to therapy , which showed a significant association (**P-value was 0.01**), this result was similar to what reported byYuan et al (2016), that was the only study in the evaluation of our target gene polymorphism in AML patients and response to therapy, they found also a significant difference between dominant model of genotypes (TA+AA) versus TT genotype, Yuan et alalso found that TT type showed more response to chemotherapy than TA and AA (P-value was **0.041**) .

InYuan et al study,DNMT3A SNP (rs11695471) was associated with reduced chemosensitivity and worse for overall survival (OS) in AML patients, OS could not assess in our study because the short follow up period of patients.

Conclusion

weconcluded from this study that DNMT3ASNP rs11695471 polymorphism is common among Iraqi AML patients and it might be a potential risk factor inAML development and might affect the response to treatment. Further studies on larger sample size and wider age range are recommended to evaluate the potential role of DNMT3A polymorphic variants in AML progression, prognosis, response to therapy and overall survival.

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