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Detection KIT D 816 Mutation and its Association with Outcome of Sudanese Acute Myeloid Leukaemia Patients at Khartoum State

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Abstract: ***Background: Acute Myeloid Leukemia (AML) is a fatal disease and have increasing prevalence in Sudan and occurs at all ages from the neonatal period to old age. It has multiple factors caused AML. One of the important these reasons the cytogenetic abnormalities what may affect the outcome in patients with AML include FLT3, KIT, CEBPA, BAALC, ERG, MLL, and NPM1.This study conducted to detection KITD 816 mutation and its association with outcome of Sudanese Acute Myeloid Leukaemia's patients at Khartoum state under chemotherapy treatment within 30 diagnosed AML patients in different types of FAB classifications were selected 14 (46%) male and 16(53%) female. The age was range from (5-70 years) during the period from May 2015 to May 2017 at Radio-Isotopcenter Khartoum, Omdurman Military Hospital, GafarIbn auf paediatric hospital. Result: this study showed significant association between frequency of KIT D816 mutations and incidence of AML disease (p.value=0.00) the percentage (76.6%) of patients positive for KIT D 816 mutation and (23.3%) of patients negative for KIT D 816 mutation. Also There was showed insignificant association between KITD816 mutation and AML patient's outcome under chemotherapy treatment as general (p.value=0.666) but there is insignificant association between KIT D 816 mutation and AML patient's outcome with (p.value=0.666) but there was significant association between adverse patient's outcome with KIT D 816 mutation if combination with some types of AML(M0, M4 and M5) comparing with control group with (p.value= 0.008).Also there was significant increased frequency in male by (43.3%) more than female (33.3%) with (P.value=0.05) and there was insignificant association between KIT D816 mutation and age of patients (P.value=0.55). Conclusion: KIT D 816 mutation had significant association with AML disease at different types and especially affected male more than female; generally it had insignificant affect in outcome of AML.***

Keywords: acute myeloid leukaemia, KIT D 816

1. Introduction

The acute leukaemias are characterized by proliferation of immature cells, either lymphoid or myeloid, with a failure of differentiation to mature end cells. Because the immature cells are proliferating in the bone marrow they replace normal haemopoietic cell. (Barbara, 2004).

Acute myeloid leukemia (AML) results from the malignant transformation of a bone marrow (myeloid) progenitor cell or stem cell, which is the normal precursor for granulocytes, erythrocytes, and megakayocytes. The traditional classification of the acute leukemias has relied on morphologic description, reflecting the predominant cell type present within the bone marrow population and relating that cell to its normal hematopoietic counterpart. This system was based solely on light-microscopic evaluation of routinely stained blood and marrow smears, supplemented by a limited number of cytochemicalprocedu- res. In 2001, a committee of the World Health Organization described a comprehensive classification scheme that utilizes morphology, immunephenoty –ping etiology, and cytogenetics and more clearly distinguishes between AML and other myeloproliferative disorders. A diagnosis of AML is established when 20% or more of the nucleated marrow cells are blast cells. Clonalchromosomal abnormalities can be detected in most cases of AML. Particular abnormalities correlate with specific morphologic subtypes and clinical profiles. These cytogenetic abnormalities are somatic (rather than germ line) mutations that frequently result from translocations of chromosomal DNA, resulting in new (abnormal) protein products from the resultant fusion genes. It is assumed that the protein products from these fusion genes are responsible for the cellular dysregulation that leads to the malignant state. Such recurring chromosomal abnormalities are critical in determining theraeutic strategy and have provided important independent. Information regarding response to therapy and overall prognosis. Genes known to affectthe outcome in patients with AML include *FLT3*, *KIT*, *CEBPA*, *BAALC*, *ERG*, *MLL*, and *NPM1*. (Mark*etal*, 2008).

It is strongly recommended that cytogenetic analysis be performed before initiation of therapy on every newly diagnosed patient because studies of the prognostic significance of recurring cytogenetic abnormalities in AML have yielded consistently similar results. Thus, in many centers, plans for postremission therapy rely heavily on cytogenetic analysis at diagnosis. Cytogenetic data have been used to map chromosomal break points at a molecular level, allowing for the use of more sensitive techniques, including probes for fluorescence in situ hybridization and primers for reverse transcriptase polymerase chain reaction. However, both of these methods test only for specific, defined genetic mutations and are not used initially for

general screening or for a comprehensive evaluation. (Mark*etal*, 2008)**.**

Acute myeloid leukaemia occurs at all ages from the neonatal period to old age. However, the incidence increases steadily. (Drew, 2003).

2. Materials and Methods

Study design
This study is analytical retrospective cohort study conducted from May 2015 to May 2017.All patients received idarubicin plus cytarabine or behenoyl cytosine arabinoside 3 + 7 induction chemotherapy treatment. Compare case and control group of study population. Aimed to detect KIT D816 mutation and its association with patient's outcome in Sudanese patients with Acute Myeloid Leukaemia under chemotherapy treatment in Khartoum.

Study area population:
This study was conducted in GafarIbn Auf hospital, Mallitary Hospital, Radio Isotope Centre Khartoum and Flowcytometery lab. The sample size of 30 venous blood samples was collected from diagnosed AML patients who are classified into two groups.one of them who KIT D 816 positive act as cases group and other who KIT D 816 negative act as control groups.

Sampling and sample method:
Individuals whom diagnosed as Acute Myeloid Leukemia were selected convenience non probability way and data collected using self –administrated per-coded questionnaire and return to hospital recorders which were specifically designed to obtain information that helped in study.

Inclusion criteria
Diagnosed de novo Acute Myeloid Leukemia patients. Confirmed cases of Acute Myeloid Leukemia patients, were under chemotherapy treatment.

Exclusion criteria
Any patients who may have other type of malignance could affect the study line. Transformed AML patients from other types of malignancies.

Data analysis
The collected data proceed for analysis using SPSS version 19 computerized program and the data presented in form of tables.

Sample collection
Two and half milliliter (ml) of EDTA anti-coagulated venous blood was collected from each patient.

Complete blood count
Complete blood count was performed by full automated hematological analyzer (sysmex –KX21N, Japan)

DNA extraction
Genomic DNA was extracted from EDTA samples by using G-Spin Total DNA Extraction mini KIT **(**G-Spin-

Detection of KIT D 816 Mutation

KIT D 816Mutation
KIT D 816 mutation was detected using Allele-specific competitive blocker polymerase chain reaction (ACB-PCR).PCR mixture 20 µL as follow 4 µL of DNA template, 1 µL from each primer Table(1) and 13 µL of D.W with master mix (premix -Interon).

Table 1: Primer's sequence design :

ACB-PCR	Primer sequence
Forward	5'- GTG ATT TTG GTA TAG CCA GAG A phosphate-3
Forward Mutant	5'- GTG ATT TTG GTC TAG CCA GAA T-3
Reverse	5-AAT CCT TTG CAG GAC TGT CAA G-3

After an initial DNA polymerase activation step (95 °C for 30 sec), 35 cycles of 94 °C, 30 s/62 °C, 30 s/72 °C, 45 s were performed with a final 72 °C extension for 5 min. The 99-bp products were visualized on an 2 % agarose gel. Stained ethidium bromide. 5 µL from PCR products and100 bp DNA ladder (Intron –Korea) were transferred on to the agarose gel and after one hour for electrophoresis the result of PCR product was detected by using gel documentation system (*SYNGENE, JAPAN)* in figure1

Interpretation:
It Gives ampilcon size 99bp compared with ladder (marker size 100 bp) That is positive reaction and any size considered as Negative result.

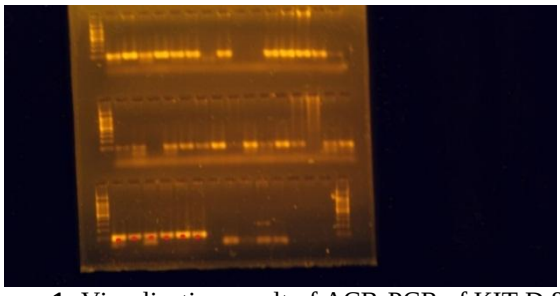

Figure 1: Visualization result of ACB-PCR of KIT D 816 mutation in gellelectropho- resis

3. Results

- **Frequency and percentage of case and control groups:** 30 diagnosed AML patients in different types of FAB classifications at RICK center, Omdurman Mallitary hospital and GafarIbn Auf paediatric Hospital and according to KIT D 816 mutation analysis divided into two groups case group who have KIT D 816 mutation and control who their free from mutation. The frequency of case group 23 patients (76%) and control group 7 patients.
- **Frequency of male and female: Sample** collected from 14 patients (46.7%) male and 16 patients (56.3%) female.
- **Age group:** conducted in age group from 5 years to 70 years.
- **Frequency and percentage of Types of AML among cases and control groups :**see table (3)
- **Frequency and percent- age of AML patient's outcome results under chemotherapy treatment:** Showed equal

value. 15(50%) patients had good outcome and 15(50%) patients with poor outcome.

- **Frequency and percentage of KIT D 816 mutation within AML patients comparing with healthy individuals:** shows significant association between frequency of KIT D 816 mutation and occurrence of AML (P.value =0.00).76% of cases positive for KIT D816 mutation and 23% negative for KIT D 816 mutation comparing with 15 healthy individuals who were gave negative results for KIT D816 polymorphism. See both Tables 4 and 5.
- **Comparison of patient's outcome between case group and control group with KIT D 816 mutation for patients under chemotherapy treatments.:** Shows insignificant association between KIT D 816 mutation with outcome of patients under chemotherapy treatment (p.value=0.666).Table 6.
- **Comparison of result KIT D 816 mutation among different age of AML patients :**Shows insignificant association between KIT D816 mutation results and different age of patients among case group (34.83x1000C/cumm±SD) and control group (41.86×1000C/cumm±SD)(P.value=0.55).
- **Comparison of frequency KIT D 816 mutation results combination with different types of AML and patient's outcome:** shows significant association between KIT D816 mutation combination with different types of AML and outcome of patients(p.value=0.008).Table 7.
- **Comparison the results of KIT D 816 mutation with gender:** shows significant association between KIT D816 mutations result with gender (p.value=0.05) by the high incidence of mutation in male (43.3%) more than female (33.3%).Table 8.

Table 2: Normal range of cells blood counts of individuals age >6 years:

Parameter	Male	Female
Haemoglobin g/L	135 - 180	115 - 160
WBC $x10^9$/L	4.00 - 11.00	4.00 - 11.00
Platelets $x10^9$/L	150 - 400	150 - 400
MCV fL	78 - 100	78 - 100
PCV L/L	0.40 - 0.52	0.37 - 0.47
RBC $x10^{12}$/L	4.5 - 6.5	3.8 - 5.8
MCH pg	27.0 - 32.0	27.0 - 32.0
MCH g/L	310 - 370	310 - 370
RDW	11.5 - 15.0	11.5 - 15.0
Neutrophils	2.0 - 7.5	2.0 - 7.5
Lymphocytes	1.0 - 4.5	1.0 - 4.5
Monocytes	0.2 - 0.8	0.2 - 0.8

Table 3: Frequency and percentage of Types of AML among cases and control groups:

Type	Frequency	Percent
M0	2	6.7
M1	3	10.0
M2	7	23.3
M3	13	43.3
M4	2	6.7
M5	2	6.7
M6	1	3.3
Total	30	100.0

Table 4: Frequency and percentage of KIT D 816 mutation within AML patients comparing with healthy individuals:

Study Group			KITD816mutation		Total
			Positive	Negative	
	Case	Count	23	0	23
		% of Total	76.7%	.0%	76.7%
	control	Count	0	7	7
		% of Total	.0%	23.3%	23.3%
Total		Count	23	7	30
		% of Total	76.7%	23.3%	100.0%

Table 5: Frequency of KIT D 816 mutation within healthy individuals

Comparison group	Number	KIT D816	
Healthy individuals	15	Positive	Negative
		0 (0%)	15 (100%)

Table 6: Comparison between patient's outcome with KIT D 816 mutation for patients under chemotherapy treatments

KITD816mutation			Outcome		Total
			Good	Bad	
	Positive	Count	11	12	23
		% of Total	36.0%	40.7%	76.7%
	Negative	Count	4	3	7
		% of Total	13.3%	10.0%	23.3%
Total		Count	16	14	30
		% of Total	53.3%	46.7%	100.0%

Table 7: Comparison of frequency KIT D 816 mutation results combination with different types of AML and patient's outcome:

Type AML			OUKIT				Total
			positive good	negative good	positive poor	negative poor	
	M0	Count	0	0	2	0	2
		% of Total	.0%	.0%	6.7%	.0%	6.7%
	M1	Count	0	0	1	2	3
		% of Total	.0%	.0%	3.3%	6.7%	10.0%
	M2	Count	6	0	0	1	7
		% of Total	20.0%	.0%	.0%	3.3%	23.3%
	M3	Count	6	4	3	0	13
		% of Total	20.0%	13.3%	10.0%	.0%	43.3%
	M4	Count	0	0	2	0	2
		% of Total	.0%	.0%	6.7%	.0%	6.7%
	M5	Count	0	0	2	0	2
		% of Total	.0%	.0%	6.7%	.0%	6.7%
	M6	Count	0	0	1	0	1
		% of Total	.0%	.0%	3.3%	.0%	3.3%
Total		Count	12	4	11	3	30
		% of Total	40.0%	13.3%	36.7%	10.0%	100.0%

Table 8: Comparison the results of KIT D 816 mutation with gender

KIT D 816 mutation	Gender		Total
	Male	Male	
Positive	13 (43.3%)	10(33.3%)	23 (76.7%)
Negative	1 (3.3%)	6 (20.0%)	7 (23.3%)
Total	14 (46.7%)	16 (53.3%)	30 (100%)

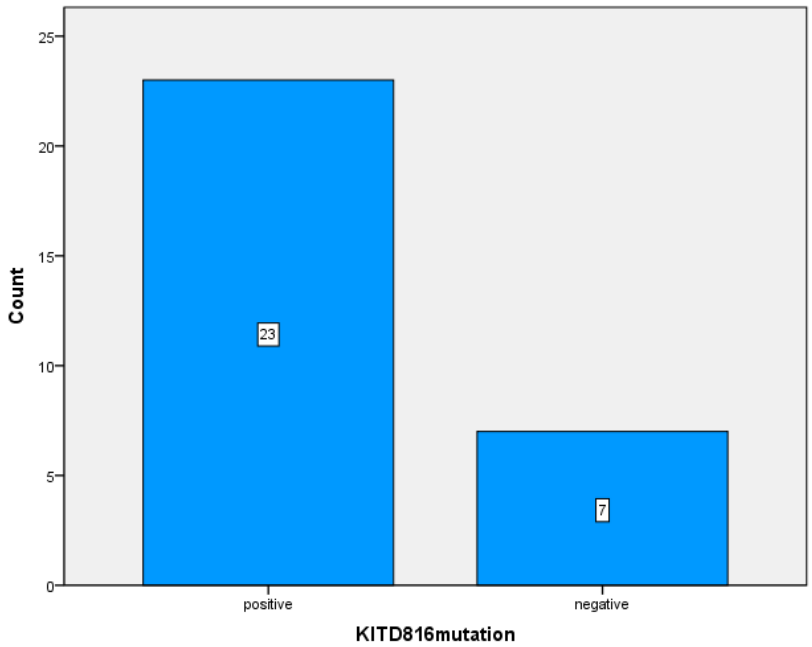

Figure 4: Frequency of KIT D 816 mutation within AML patients

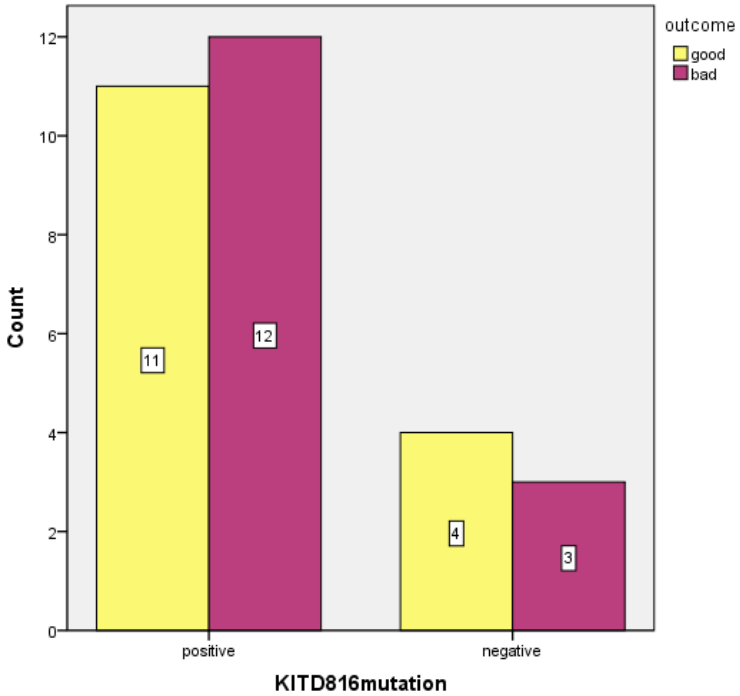

Figure 5: Comparison of KIT D 816 mutation with outcome.

4. Discussion

This is retrospective cohort study was conducted in Radio isotope center, Omdrman Military hospital and GafarIbn auf paediatric hospital during the period of May -2015 to May 2017.The study included 30 patients, 14 male and 16 female with Acute Myeloid Leukemia in different FAB classification (M0, M1, M2, M3, M4, M5and M6) classified into two groups :case group and control group. Also we have 15 samples from healthy individuals as technical control. The age was range from (5-70 years) and duration of disease from 6 months to 2 years.

By ACB-PCR technique this study showed significant association between frequency of KIT D816 mutations and incidence of AML disease (p.value=0.00) the percentage (76.6%) of patients positive for KIT D 816 mutation and (23.3%) of patients negative for KIT D 816 mutation and this result dissimilar to a study (Susanne *et al*, 2005)which has been carried on 1940 AML patients randomly, showed insignificant association between frequency of KIT D816 mutation and incidence of AML.

According to analysis results of KIT D 816 mutation by Allel Specific Competitive Bloker PCR assay classified the patients into two groups: Case group whose were positive for KIT D816 mutation contains 23 patients and control group whose were negative for KIT D 816 mutations that contains 7 patients'. All patients in both groups received idarubicin plus cytarabine or behenoyl cytosine arabinoside

3 + 7 induction chemotherapy treatment. Then follow up all patients in case and control groups via CBC test results within minimum period 4 months and more and clinical findings to evaluate patient's outcome.

There was showed insignificant association between KITD816 mutation and AML patient's outcome whose were under chemotherapy treatments as general (p.value=0.666) and this result agree with report (Jesica*et al*, 2009) in pediatric AML patients but disagree to study (Roberto*et al*, 2005) within Italiano AML adult patients.

Also this study showed significant association of KIT D816 mutation when was combined with some types of AML and patient's outcome (p.value=0.008).in this study KIT D 816 mutation when present with M0, M4 and M5 can lead to adverse patient's outcome; in spite of (M4) one of favourable outcome factors in AML; in another hand KIT D 816 mutation when present with M2 and M3 had good outcome by comparing with patient's outcome of control group whose were free from mutation and this result was also in corresponding with the results of (Schnittger *et al*, 2006) who had defined the reason in KIT D816 variation stabilizes the active kinase state which lead to causes ligand–independent constitutive phosphor- rylation and activation of KIT, leading to uncontrolled growth, so imatinib is unable to bind and inhibit KIT therefore patients can't response to chemotherapy treatment and then caused adverse outcome .

Also there is significant association between frequency of KIT D 816 mutations and gender which is affected male patients more than female when comparing with control group that frequency of female patients free from mutation more than frequency of male patients whose were negative KIT D816 mutation (p.value=0.05). And there was insignificant association between frequency of KIT D816 mutation and age (p.value=0.55).

5. Conclusion

KIT D 816 mutation is highly frequent in AML patients at different types and especially affected male more than female, generally it had insignificant affect in outcome of AML patients but showed significant association with adverse outcome if KIT D 816 mutation presence with some types of FAB classification of our AML patients AML such M0, M4 and M5.

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