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Research Article

Detection of RUNX1-RUNX1T1 Fusion Gene in AML Patients by FISH Technique in Iraq

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I N F O

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A B S T R A C T

Background: AML with t(8;21)(q22;q22.1) is a balanced translocation that results in the fusion of *RUNX1* and *RUNX1T1*. The translocation product is located on derivative chromosome 8. It has characteristic morphologic and immunophenotypic features and is linked with good prognosis. t(8;21)(q22;q22.1) AML is diagnostic of AML regardless of blast count.

Objectives: To detect the *RUNX1-RUNX1T1* fusion gene) in patients with AML by FISH technique and to investigate the relation between this chromosomal abnormality and the immunophenotypic markers CD117, cMPO, CD34, CD13, CD64 and CD33, clinical features and haematological parameters (Hb, WBC, blast percentage and platelets).

Materials ad Methods: Fifty patients with de novo AML were selected sequentially from Baghdad teaching hospital in medical city from June 2020 till April 2021. History was taken and data were collected for each patient using a questionnaire form that included: name, age, sex, symptoms and physical signs. The data of haematological parameters and CD markers expression were collected from the patients' diagnostic reports.

Results: The *RUNX1/RUNX1T1* fusion gene expression was positive in 4 patients representing 8% whereas 46 patients representing 92% of the samples had negative gene expression. Among the 4 positive AML patients for *RUNX1*/*RUNX1T1* fusion gene expression, M2FAB subtype was revealed in all the cases. All the positive cases expressed CD117 and CD34 markers, while it was noted that 3 out of 4 positive cases were having cMPO and CD13 markers and one positive case expressed CD33 and CD64.

Conclusion: It's shown that *RUNX1-RUNX1T1* fusion gene frequency in AML Iraqi patients is similar to international reports.

Keywords: Acute Myeloid, Leukaemia, Translocation, FISH, t(8; 21), RUNX1-RUNX1T1

Introduction

Acute Myeloid Leukaemia (AML) with recurrent genetic abnormalities is connected to distinctive clinicopathological features having prognostic significance and is considered the most powerful predictors of treatment outcome. t(8;21) is considered as the commonest specific translocation available.[1]In this type of translocation, there is neutrophil lineage maturation. There are large myoblasts with abundant basophilic cytoplasm that contain azurophilic granules in the peripheral blood as well as in the bone marrow. *RUNX1-RUNX1T1* fusion gene induces cell differentiation, renewal capacity and proliferation. It also inhibits the core-binding factor that plays a key role in early haematopoiesis. It has been found that *RUNX1- RUNX1T1* and core-binding factor targeting are not enough to induce leukaemia. More additional mutations are needed to trigger leukaemogenesis. [2]

Diagnosis of this type of recurrent translocation needs either Fluorescence In Situ Hybridization (FISH) or Polymerase Chain Reaction (PCR) and both techniques are still not used as routine diagnostic practice in Iraq. Diagnosis depends only on FAB classifications based on morphology in Iraq. In this study, we studied the frequency of *RUNX1-RUNX1T1* fusion gene in a sample of AML Iraqi patients by using the FISH technique as an attempt to clear up the exact role of this genetic aberration in the AML subtype.

Materials and Methods

This cross-sectional study was conducted from June 2020 till April 2021 and was approved by the institutional review board of medical college/ Al-Nahrain University. Work was done in the post graduate lab of Pathology and Forensic Medicine Department and Medical Researches Unit in Al-Nahrain College of Medicine. Informed consent was taken from each participating patient. Data were collected for each patient using a questionnaire that included: name, age, sex, main symptoms, and physical signs. The data regarding haematological parameters and CD markers expression were collected from the patients' diagnostic reports. Fifty patients with de novo AML were selected sequentially from Baghdad teaching hospital in the medical city.

Patients' criteria of inclusion were: a new diagnosis with AML by immunophenotype with an age of more than or equal to 18 years old. Also, patients were not known to have any other malignant disease or have taken any chemotherapy. The diagnosis depended on immunophenotyping as well as morphology of peripheral blood and bone marrow samples by haematopathologists in the laboratories. Sodium heparinized peripheral blood sample of 3 ml was collected and labelled with the patient's age, name, and date of collection. After centrifugation, the specimens were stored as a fixed pellet at 4°C till the start of FISH studies. FISH was done by using a dual colour, directly labelled *RUNX1/ RUNX1T1* fusion probe to reveal the signal of fusion gene in cells containing t(8;21). In cells with t(8;21), the yellow fusion signal representing *RUNX1-RUNX1T1* includes a DNA sequence that hybridizes at (q22; q22.1) as shown in Figure 3.

Statistical analysis was done using Microsoft Excel program and SPSS. P-value < 0.05 was intended to be statistically significant.

Results

RUNX1/RUNX1T1 fusion gene expression was positive in 4 patients representing 8% whereas 46 patients representing 92% had negative gene expression, as shown in Figure 1. Regarding gender, there were 3 male patients who were positive 75%, while in the female group of patients, 1 patient 25% was positive. The mean age of positive patients for *RUNX1/RUNX1T1* fusion gene expression was 52.75 years, whereas, for the negative fusion gene expression group of patients, it was 57.61 years (P= 0.54). Table (1).

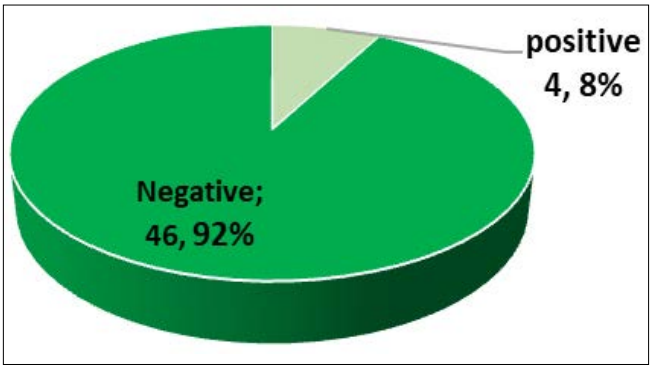

Figure 1.t(8;21) Fusion Gene Expression of AML Cases involved in Study

Four patients came at the time of initial diagnosis. Symptoms at the time of presentation included weight loss, recurrent infection, easy bruising, and fatigue. Regarding the haematological parameters, the mean value of WBC count in patients with positive expression for *RUNX1/ RUNX1T1* fusion gene was 38.25X10^9/L, whereas it was 40.75 10^9/L (p = 0.70) in the negative group. The mean value of Hb in the positive group was 9.6g/dL. On the other hand, it was 8.6 g/dL in the negative group (P = 0.39). The mean value of platelet count in *RUNX1/RUNX1T1* positive gene expression patients was 37.25 X10^9/L, while it was 52.48X10^9/L (P = 0.70) in the gene expression negative patients. Bone marrow blast cells mean value percentage in the fusion gene expression positive group was 49.25%. On the other hand, it was 52.83% (P = 0.67) in the gene expression negative group as shown in Table 1.

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Table 1.Comparison of Parameters according to *RUNX1/RUNX1T1* Fusion Gene Expression in AML Patients

	Positive N 4 (Mean ± SD)	Negative N 46 (Mean ± SD)	P-value
Age (year)	52.75±16.3	57.61±15.1	0.54
Hb (g/dL)	9.6±2.72	8.63±2.1	0.39
WBC count (*10^9/L)	38.25±15.8	40.75±12.5	0.70
Blast (%)	49.25±18.46	52.83±116.16	0.67
Platelet count (*10^9/L)	37.25±9.8	52.48±19.1	0.12

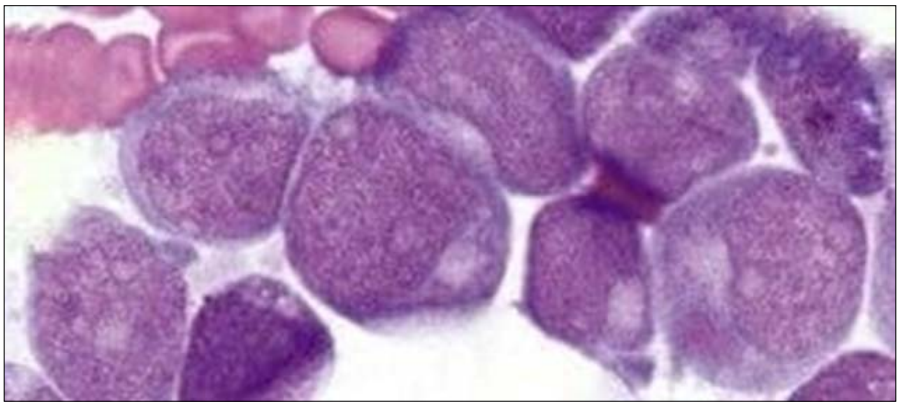

Figure 2.(Case 2) t(8;21)(q22;q22q22) AML. Smear of Bone Marrow Aspirate showing Auer Rods in Blasts. Granulocytes with Pink Coloured Granules are seen in the Background (Wright-Giemsa stain ×1,000)

Table 2.Morphological, Immunophenotypic and FISH Diagnostic Findings

Case No.	Blast Morphological Features	FAB Type	Immunophenotype	FISH
1.	Pink granules, basophilic cytoplasm, no Auer rods	M2	CD13- , CD64+, CD33+, CD34+, CD117+, MPO+	+
2.	Pink granules, basophilic cytoplasm, Auer rods	M2	CD13+, CD64-, CD33- , CD34+, CD117+, MPO+	+
3.	Pink granules, basophilic cytoplasm, no Auer rods	M2	CD13+, CD64- , CD33- , CD34+, CD117+, MPO+	+
4.	Pink granules, basophilic cytoplasm, Auer rods	M2	CD13+, CD64- , CD33- , CD34+, CD117+, MPO-	+

Summary of the immunophenotypic and morphologic findings is available in Table 2. Bone marrow aspirate smears displayed blasts with basophilic cytoplasm and salmon pink granules in all the cases. Long Auer rods with large granules were seen in case no. 2 and 4 (Figure 2). Cellularity of 40%-90% is shown in the bone marrow biopsy samples with a remarkable increase in the immature myeloid elements.

The flow cytometry analysis of immunophenotype displayed that blasts of all cases are expressing CD117 and CD34. Myeloperoxidase and CD13 were positive in 75% of the positive cases for *RUNX1/RUNX1T1* fusion gene expression, while CD33 and CD64 markers were expressed in case 1.

FISH analysis was performed on interphase nuclei revealing two RUNX1T1 signals, one signal on the normal while the other one on the abnormal chromosome 8, two RUNX1 signals, one on the normal and the other one on the abnormal chromosome 21, with a single RUNX1-RUNX1T1 fusion signal in 60 of 100 nuclei examined (Figures 3, 4, and 5).

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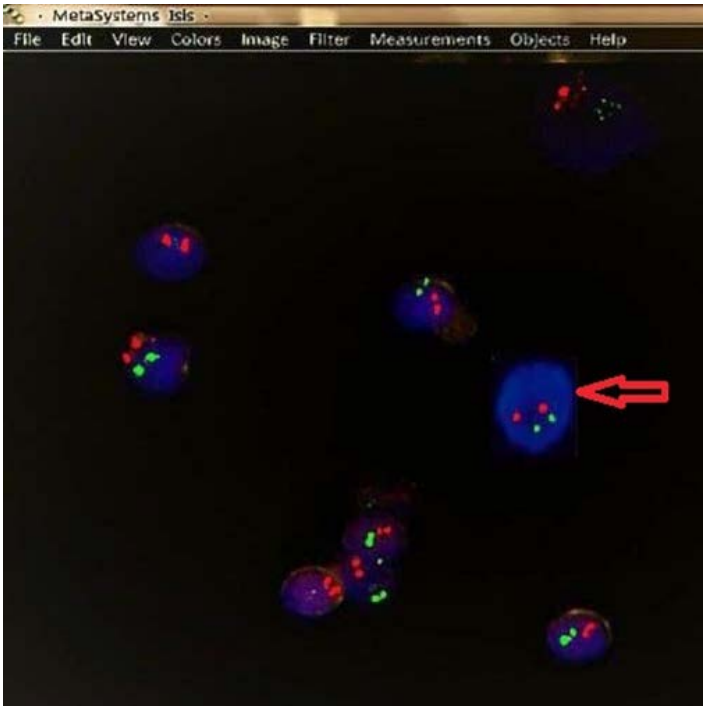

Figure 3.FISH Analysis with the LSI RUNX1/RUNX1T1 Dual Colour Fusion Translocation Probe on a Peripheral Blood Cell Sample, showing Leukaemic Cells without Fusion Gene Expression. It shows Two Green Signals representing RUNX1T1 and Two Orange Signals representing RUNX1 (Red Arrow)

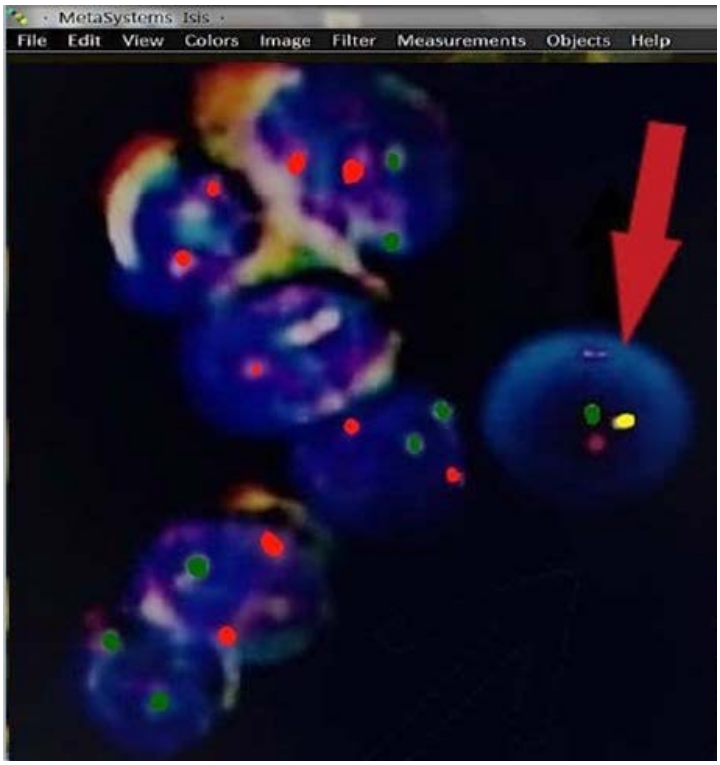

Figure 4.FISH Analysis using LSI Dual/ Colour Probe for Interphase Detection of Gene Fusion, showing Leukaemic cells where the Probe with the Orange-label spans the Breakpoint at 21q22.1 RUNX1and the Probe with the Green-label spans the Breakpoint at 8q21.3-22.1 RUNX1T1. The translocation (8;21) results in RUNX1- RUNX1T1 Fusion on the Derivative Chromosome 8 (Yellow Signal marked by Red Arrow). To simplify its identification, Chromosomes are Counterstained by DAPI on power 60X

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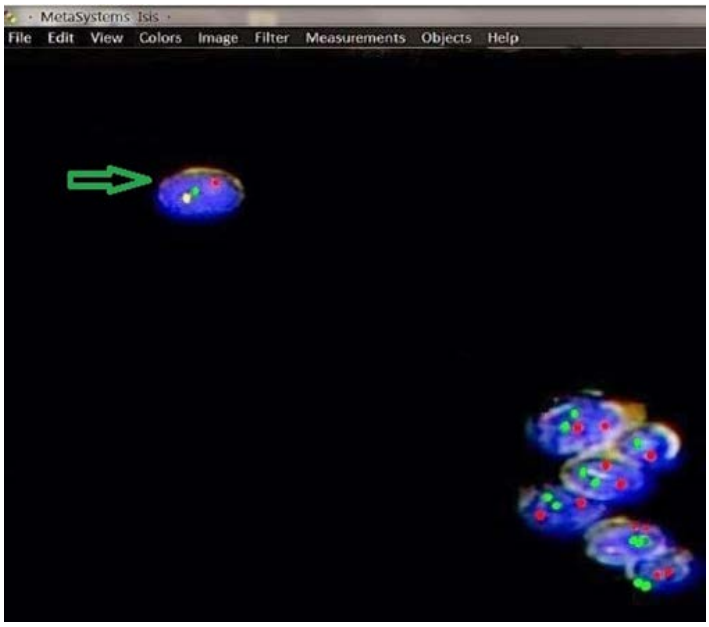
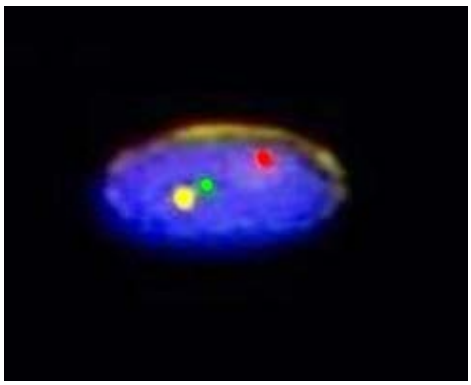


a



b

Figure 5.(a) FISH Analysis using LSI *RUNX1*/*RUNX1T1* Dual Colour Fusion Translocation Probe for Interphase Detection of Gene Fusion, showing Leukaemic Cells without Fusion Gene Expression, Two Green Signals representing *RUNX1T1* and Two Orange Signals representing *RUNX1*. The Yellow Signal indicated by the Green Arrow represents Fusion Gene *RUNX1*/*RUNX1T1* (b). Zoomed in Image of the Leukaemic Cell with Yellow Signal representing Fusion Gene *RUNX1*/*RUNX1T1*

Discussion

t(8;21)(q22;q22) AML is realised as a specific type of AML according to the WHO classification.[3] It is more frequent in younger patients and is linked to a favourable outcome.[4] The leukaemic cells have distinctive morphologic features that include pink coloured granules of the cytoplasm with long Auer rods. Most of the cases usually are within AML-M2 category of FAB classification.

RUNX1/RUNX1T1 fusion gene was detected in 4 out of 50 patients (8%) by using FISH technique. This result is close to a study in Iraq that reviewed *RUNX1/RUNX1T1* mutation among 134 patients with AML by using filter paper cards in 2014 and the result was 14.2%.[5] On the other hand, a study in Egypt involving 100 patients revealed that *RUNX1/RUNX1T1* fusion gene is present in 45.7% of the patients by using a different technique.[6] Here, the frequency of occurrence of *RUNX1/RUNX1T1* transcript is higher than the one observed in the current study. This difference may be due to diversity in age group and population structure involved in that study in addition to the difference in samples' size and the technique used. On an international level, the result of the current study goes with a study that took place in Germany. It showed that *RUNX1/RUNX1T1* is present in 6% out of 916 patients detected by using PCR.[7]

According to the morphology, all the cases showed morphologic features of the classical t(8;21)(q22;q22) AML, as well as classified as AML-M2 according to FAB criteria. The mean age of the positive expression group

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was 52.7 years which is lower than the negative expression group that was 57.6 years. Age distribution of the two groups was statistically not significant (P = 0.54). The result mentioned above does not go with other data available.[5,6] The discrepancy in results may be attributed to small sample size and difference in population structure. Regarding gender, the ratio of males to females in *RUNX1/RUNX1T1* positive group was 3:1. The current result agrees with other data.[5,8,9]

Among the 4 positive AML patients for *RUNX1/RUNX1T1* fusion gene expression, M2 FAB subtype was revealed in all the cases (100%). The result in the current study is in agreement with that of other studies.[7,10]

The commonest sign and symptom was pallor in both positive and negative groups for *RUNX1/RUNX1T1* fusion gene expression, followed by fever also in both groups. Lethargy and bleeding percentages in the positive group were higher than in the negative group. Less frequent signs and symptoms were weight loss, hepatosplenomegaly, and LAP which were higher in the positive group. These results are similar to that of other studies.[11] On the other hand, another study available showed that fever, weakness, and body pain were more common features.[10]

About the haematological parameters, the mean value of WBC in a positive group is almost the same as in a negative group. There was no significant difference between positive and negative fusion gene expression groups (P = 0.70) Table 1. In another study, the mean of WBC was close to that obtained by the current study.[12] In the current study, the mean Hb level in the positive group was close to that seen in the negative group, statistically not significant. This agrees with other study data which showed that all cases presented with anaemia.[13] The mean level of platelets in the positive group was much lower than the mean level in the negative group and it was statistically not significant. This result goes with another study which showed that all cases presented with thrombocytopenia.[13] Regarding the BM blasts, the mean value in a positive expression fusion gene group was lower than the mean level in a negative group and it's statistically not significant. This result agrees with another international study which shows a similar result.[13]

All the positive cases of *RUNX1/RUNX1T1* fusion gene expression expressed CD117 and CD34 markers (100%). This is in agreement with another study.[14] However, it was noted that 3 out of 4 cases with positive *RUNX1/RUNX1T1* fusion gene expression had cMPO and CD13 markers (75%) and one case expressed CD33 and CD64 (25%). Studies described a more favourable prognosis to cases in which myoblasts demonstrate co-expression of cMPO, CD33, and CD117.[14,15]

Conclusion

Our work showed that RUNX1-RUNX1T1 fusion gene frequency in AML Iraqi patients is similar to international reports, with a similar morphological and immunophenotypic pattern.

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Conflict of Interest: None

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