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Detection of AML1-ETO Fusion Gene in Iraqi Patients with Acute Myeloid Leukemia using Nested PCR and Flow Cytometry

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Abstract
The rearrangement and instability of genomic material causes the occurrence of fusion genes. Over 800 distinct fusion genes have been found in human cancer, most observed in hematological cancers. Chromosomal rearrangements such as deletion, inversion, translocation, and amplification are the primary forms that lead to fusion gene formation, which are ultimately resulting from aberrant DNA transcription. The purpose of this research was to use nested polymerase chain reaction (PCR) and flow cytometry to determine how frequent the AML1-ETO fusion gene is among Iraqi AML patients. The AML1-ETO fusion gene was detected and documented in twenty-seven percent (27%) of acute myeloid leukemia (AML) patients were found to have the AML1-ETO fusion gene. The results of this study add to our understanding of the prevalence of the AML1-ETO fusion gene in Iraqi AML patients and have implications for the development of more effective treatment options.

Keywords
Acute myeloid leukemia, Chromosomal rearrangement, Fusion gene, AML1-ETO

INTRODUCTION

Cancer is a result of genomic material rearrangement and instability. This rearrangement leads to a novel fusion gene that links two distinct genes. The term "fusion gene" gained attention among scientists after discovering the Philadelphia chromosome in 1960 (Roy et al., 2017). With the discovery of the Philadelphia chromosome, a door was opened for a new era of accurate detection and better treatment options for cancer patients (Dong et al., 2020; Kim & Zhou, 2019). With technology development, fusion genes are easily detected and considered a hallmark of cancers (Blundell et al., 2007; Cowell & Austin, 2012). One of the best prognostic indicators in AML is the presence of the onco-fusion protein AML1-ETO, which is produced by the most prevalent chromosomal translocation, t (8;21) (Liu et al., 2018). The t (8;21) translocation is thought to be the first step in the progression of AML (Chen et al., 2021). When the AML1 gene is rearranged with the ETO gene, a fusion protein is produced that includes the first 177 amino acids of AML1 and almost the entire length of the ETO protein (Yang et al., 2018; Chen et al., 2021). Essential for both fetal and adult haematopoiesis, AML1 acts as a binding transcription factor for DNA. ETO, also known as 821, is a corepressor that works by inducing the NCoR/SMRT/HDAC complexes to form (Yang et al., 2018). The binding domain for DNA of AML1 is joined with the ETO gene four conserved domains to form AML1-ETO. Although AML1-ETO was first classified as a transcriptional repressor, this does not fully capture its biological roles (Tijchon et al., 2019). The AML1-ETO onco-fusion protein has been shown to induce transcription through a mechanism involving p300 contacts (Haas et al., 2019). The absence of human investigations has made it difficult to pinpoint the precise molecular and biological pathways through which AML1-ETO begins leukemia (Mi et al., 2021). AML1-ETO's significance in the development of AML has been extensively investigated (Xiaoyan Chen et al., 2021). The primary aim of this research is to use nested polymerase chain reaction (PCR) and flow cytometry to document the percentage of Iraqi patients with acute myeloid leukemia (AML) that have the AML1-ETO fusion gene.

METHODOLOGY

Sample Collection

The period of time for this research was from May 5, 2021 to July 23, 2022 in Wasit, Iraq. Patients between the ages of 25 and 45 with acute myeloid leukemia (AML) provided the fresh blood samples. Control blood samples were also taken from healthy individuals within the same age range. A four ml of fresh blood was collected from each AML patient and healthy individual in EDTA tubes, which were labeled accordingly. The collected samples were then equally divided into two 2 ml EDTA tubes. One tube was used for RNA extraction, while the other was used for flow cytometry assay to detect CDs for further detection of fused protein presence. All the aforementioned steps were carried out within 24 hours to ensure accurate results.

Fusion Gene Detection

RNA was isolated from blood samples of Iraqi AML patients to study the prevalence of the AML1-ETO fusion gene in this population. The RNA samples were then reverse transcribed to cDNA using an applied biosystem kit. The resulting cDNA samples were used as templates for targeted primers specific to the fusion gene of interest. The samples were then amplified using a multiplex PCR procedure based on van Dongen et al. (1999). The amplification process and specificity of DNA were checked using agarose gel electrophoresis, and the results were visualized using a UV transilluminator with a 320 nm wavelength. Additionally, flow cytometry technique was employed to investigate the presence of the fusion gene at the protein level using conjugated antibodies (CD34 and CD56) as further detection methods in the study.

Statistical Analysis

Results from the research were analyzed statistically using GraphPad Prism version 9.4.1. The analysis involved calculating the percentage of positive results for the control and AML patient samples obtained through agarose gel electrophoresis. The gel electrophoresis was used to detect the fusion gene via nested PCR. In addition, the results of the flow cytometry assay were analyzed by converting the signals to digital using specialized computer software designed for the flow cytometry machine.

RESULTS AND DISCUSSION

AML1-ETO Fusion Gene Detection

A chimeric gene termed the AML1-ETO fusion gene is formed when the AML1 gene (acute myeloid leukemia 1) on chromosome 21 fuses with the ETO gene (eight twenty one) on chromosome 8 (Ross et al., 2004). Studies by (Chen et al., 2021; Lu, 2021; Wu et al., 2015; van Dongen et al., 1999) indicated that activating mutations in tyrosine kinase receptors, such as the C-KIT receptor for stem cell factor, are known to cause leukemia. Therefore, in addition to epigenetic dysregulation of gene transcription, activation of proliferative signaling pathways is required for leukemia to develop in AML1/ETO-bearing cells. The detection of ETO-AML1 in acute myeloid leukemia patient samples with Nested PCR was carried out by binding the primers as following; the AML1-A\ ETO-B primer for the first thermocycle run, and the AML1-C\ETO-D for the second thermocycle run. Results detected by gel electrophoresis showed in (Figure 1) in which the product size detected about 370 bp.

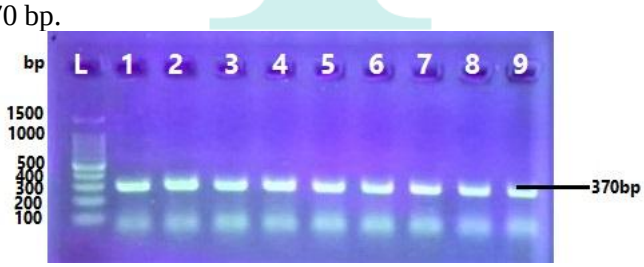

Fig. 1 AML1-ETO fusion gene positive results in AML patients detected with agarose gel electrophoresis. (L): Ladder & lane 1-9: positive result samples (product size 370bp)

Nevertheless, the Figure 2 below shows the frequency of AML1-ETO fusion. The result showed that this fusion was detected in 19 AML patients, making a frequency percentage of 27.14%.

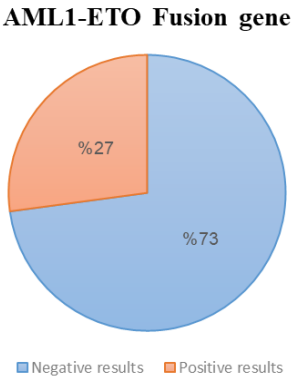

Fig. 2 Pie chart for frequency percentage of AML1-ETO fusion gene in this study where negative result represents 73% and positive result represents 27% of all studied cases

The results of our study showed a high frequency of AML1-ETO fusion gene, consistent with previous research by Kim & Zhou, 2019; Lyu et al., 2017; Wiggins and Stevenson, 2020, who reported that AML1-ETO is the most common fusion gene in AML-M2 subtype, with a prevalence of 20-40% in cases. Additionally, these studies reported that AML1-ETO may also be present in uncommon cases of AML-M1 and AML-M4 as well as therapy-related AML (t-AML). Nevertheless, in recent studies by Wiggins & Stevenson (2020), they approved the prementioned results and stated that the AML1-ETO fusion gene is present in 12-15% in AML in general and in 40% of AML-M2 subtype in specific. Moreover, the AML1-ETO fusion gene is commonly associated with a favorable prognosis and is particularly responsive to certain treatment drugs, such as cytosine arabinoside. Additionally, the van Dongen et al. (1999) findings have enabled the implementation of risk-adapted therapy based on molecular genetics and cytogenetic assessments.

AML1-ETO Fusion Gene Flow Cytometry

The detection AML1-ETO fusion gene was further conformed with flow cytometry assay in which for this fusion it recorded a high immunophenotype expression of CD 34 and CD56, as shown in (Figure 3) all positive samples were higher than 20% cell count for CD34 for CD56 as well compared with control that showed a negative result indicated less than 20% cell count.

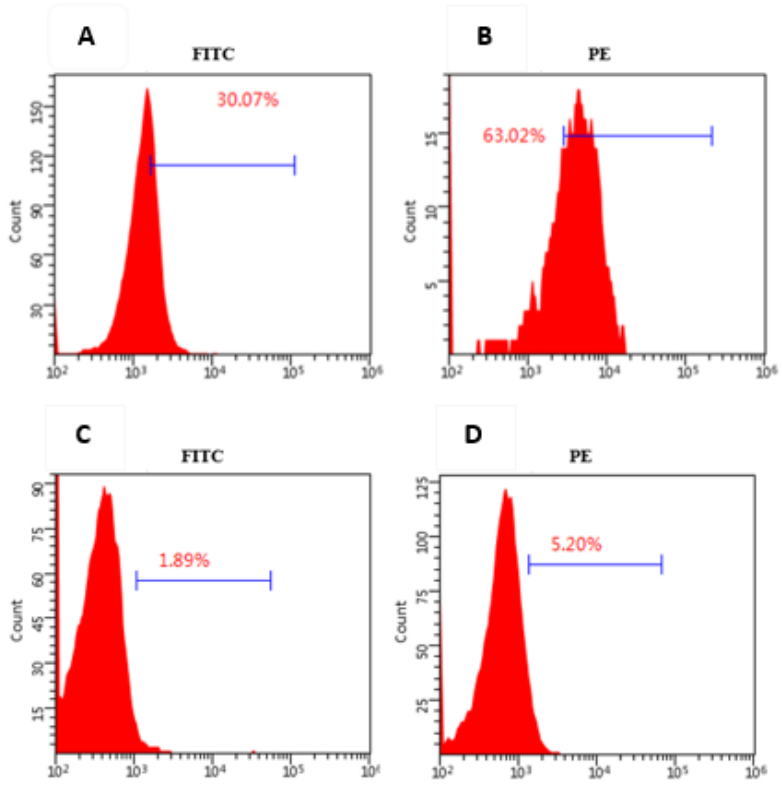

Fig. 3 Flow cytometry histogram graph showing results for conjugated antibodies of CD34 and CD56, (A and B) representing positive results for CD56 and CD34 while (C and D) representing negative results of CD56 and CD34 respectfully

In this investigation, surface marker expression of AML leukemic cells was analyzed. The results revealed a notable increase in the number of CD34+ and CD56+ cells within the leukemic cell population. These findings are consistent with prior research conducted by Yuan et al., 2001; Mori et al., 2002; Xue Chen et al., 2021, which indicated high expression of AML1-ETO for both CD34 and CD56. Additionally, Mulloy et al., 2002 conducted a study that supports our results, as they observed an increase in the overall number of CD34+ cells in the nonadherent fractions of the AML1-ETO transduced population.

CONCLUSION

In conclusion, the study aimed to determine the frequency of AML1-ETO fusion gene in Iraqi AML patients, focusing on the most common fusions reported in previous studies. The results obtained provide valuable information on the type of fusion present in AML cases, which could help in developing effective therapeutic strategies. Additionally, the method used to detect AML1-ETO fusion gene could serve as a new and reliable diagnostic approach for leukemia, which is cost-effective and easily accessible. Overall, this study highlights the significance of molecular testing for accurate diagnosis and treatment of AML patients.

AUTHORS CONTRIBUTIONS

All authors had significant roles in the conception and design, data collection, or interpretation and analysis of the study, contributed in writing the article or revising it objectively for important logical content, approved the final version for publication, and accepted full responsibility for the study's results and dissemination.

ETHICAL APPROVAL

The collection of blood samples from AML patients was performed in accordance with the ethical regulations, with number 13398 in 2016 paragraph 13 stated by the Iraqi Ministry of Health and with the consents of the patients under the direct supervision of medical staff.

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