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Frequency of PML-RARA and CBFB-MYH11 in Iraqi Patients with Acute Myeloid Leukemia

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ABSTRACT

Leukemia is a type of cancer that can be extremely aggressive, developing quickly and wreaking havoc on the body. This happens when genetic defects cause blood cell-producing cells in the bone marrow to divide uncontrollably. Because leukemia is heavily influenced by genetics, understanding the genetic material rearrangements that because cancer is key to improving treatment strategies. That's where this study comes in: by using advanced techniques like nested PCR and flow cytometry, researchers aimed to determine the frequency of two common fusion genes, PML-RARA and CBFB-MYH11, in Iraqi patients with acute myeloid leukemia. The results were striking, with PML-RARA showing a frequency of 10.00% and CBFB-MYH11 following closely behind with 5.71%. This groundbreaking research sheds light on the type of leukemia affecting Iraqi patients and could help pave the way for new and improved treatment options.

Key words: Acute myeloid leukemia, chromosomal rearrangement, Fusion gene, PML-RARA, CBFB-MUH11, translocation

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INTRODUCTION

Acute leukaemia is an illness that spreads quickly [1]. Acute leukaemia caused by DNA errors is characterised by unregulated cell division of hematopoietic stem cells [2]. In general, genetics has a significant impact in the development of leukaemia. Cancer is produced as a result of genetic material instability and rearrangement; these rearrangements are caused by translocation, amplification, deletion, and inversion [3]. All of this leads to incorrect DNA transcription, which results in fusion genes, which are defined as chimeric genes created by the combination of two distinct genes [1]. We previously claimed that fusion genes were most commonly found in haematological malignancies [4]. As a result, fusion genes were discovered in acute myeloid leukaemia (AML) (e.g., PML-RARA and CBFB-MYH11 fusion genes) [5]. The t(l5; 17) translocation is a well-balanced reciprocal translocation that results in the development of a functioning chimeric gene: PML-RARA is expressed in 100% of APL patients [6]. The PML-RARA fusion gene is the product of a translocation between the RARA gene on chromosome 17 and the PML gene on chromosome 15 [7]. Both a dominant-negative and a gain-of-function effect contribute to PML-RARA's carcinogenic activity [8]. At the promyelocytic stage, PML-RARA inhibits myeloid differentiation [7]. In addition, around 4% of individuals with de novo acute myeloid leukemia (AML) have the pericentric balanced translocation t(16;16), with inversion being far more prevalent (95%) than translocation (5%) [9]. Core Binding Factor Beta Subunit (CBFB) at 16q22 and smooth Muscle Myosin Heavy Chain 11 (MYH11) at 16p13.1 fuse to generate a chimeric CBFB/MYH11 protein in both cytogenetic abnormalities [10]. AML patients with a t(16;16) mutation have been documented in all age categories, but the majority of patients are quite young, with a median age of around 35 years [11]. Common chromosomal translocations that lead to CBFB-MYH11 fusion genes in the leukemia-initiating process are responsible for the development of CBF leukemias, a subtype of AML [10]. About half of CBF AML patients had a fusion between the CBFB and MYH11 gene

caused by t (16;16), which accounts for 12% of pediatric and 7% of adult AML [12]. This fusion occurs in the founder clone, is maintained throughout the illness, and is present in relapses, making it essential for the development of leukemia [13]. Patients with de novo t(16;16) AML have highly recurrent chromosomal breakpoints, with 80% to 90% having the type A CBFB-MYH11 fusion variant [14]. Therefore, the main objective of this study was to assess the occurrence rate of PML-RARA and CBFB-MYH11 fusion genes, which are commonly found in Iraqi patients with acute myeloid leukemia, through the utilization of nested PCR and flow cytometry techniques.

METHODOLOGY

Sample Collection

This study, was conducted in Wasit, Iraq from 5 May 2021 to 23 July 2022. The fresh blood samples were collected from Acute Myeloid Leukemia (AML) patients aged between 25 and 45 years old. Control blood samples were also taken from healthy individuals within the same age range. A total of 4 ml of fresh blood was collected from each AML patient and healthy individual in EDTA tubes, which were labelled 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. The collection of samples was performed in accordance with the ethical regulations, numbered 13398 in 2016 paragraph 13 stated by the Iraqi Ministry of Health which suggested that must take oral and written approval of the patients and under the direct supervision of medical staff.

Fusion Gene detection

The fusions PML-RATA and CBFB-MYH11 investigated for its frequency among Iraqi AML patients. RNA from blood samples were extracted then reversed transcripted to cDNA using applied biosystem kit. The cDNA samples were used as template for targeted primer of the fusion gene understudy, Samples were amplified using multiplex PCR, procedure followed was according to van Dongen et al [15]. Aamplification process, and specificity of DNA was checked with agarose gel electrophoresis, the results were shown using UV transilluminator with 320 nm wavelength. Furthermore, flow cytometry technique used to investigate fusion genes at protein level as further detection method using conjugated antibodies recorded for targeted fusion in the study which are CD34 and CD56.

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

PML-RARA fusion gene

According to Mannan et al the two genes involved in the PML-RARA fusion are both the RARA gene on chromosome 17 (which encodes the retinoic acid receptor) and the PML gene on chromosome 15 (which codes for a putative novel transcription factor) [16]. When the PML and RARA genes are switched, a chimeric PML-RARA fusion transcript is produced, and it is related with the pathogenesis of acute promyelocytic leukemia (a unique acute myeloid leukemia subtype), and it is seen in 100% of APL patients [17]. The PML-RARA fusion gene was detected using nested PCR by binding fusion primers as following; PML-A1\PML-A2\RARA-B for the first thermocycle run and PML-C1\PML-C2\RARA-D for the second thermocycle run and the results was shown by agarose gel electrophoresis the product size number detected was about 300 bp as shown in (Figure 1).

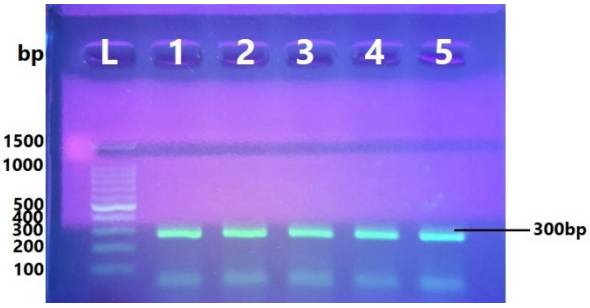

Fig 1. Agarose gel showing positive results detected with nested PCR for PML-RARA fusion gene. (L): ladder & lane 1-5: positive samples for the PML-RARA fusion (product size 300bp)

By preventing apoptotic cell death, the PML-RARA inhibits the final differentiation of hematopoietic precursor cell lines and stimulates cell proliferation 18, hence explains the aggressive behaviour and high frequency of this chimeric fusion, which in this study was detected in seven AML patients representing a frequency of 10.00% (Figure 2).

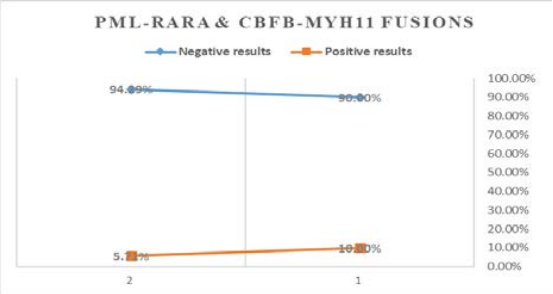

Fig 2. Frequency percentage of PML-RARA & CBFB-MYH11 fusion genes.

The PML-RARA recorded in seven patient's cases which was notably higher than the CBFB-MYH11 fusion, this frequency was agreed by Dolz et al where they suggested that this fusion is the second highest in its frequency after AML1-ETO fusion in Acute Myeloid Leukemia (AML)8, although it was reported by Liquori et al to have higher incidence percentage than it already obtained19. Attempts have been made to link the specific PML-RARA transcript type with either diagnostic or therapeutic outcomes in light of the observation that PML-RARA transcript are present in nearly all cases of APL, favoring the use of PML-RARA transcript as a PCR target for the diagnosis and follow-up detection of APL cells [20].

CBFB-MYH11 fusion gene

Pathogenesis includes inducing differentiation defects during both primitive and definitive hematopoiesis when the Core Binding Factor Beta subunit (CBFB) gene on chromosome 16q22 fuses with the smooth muscle Myosin Heavy Chain (MYH11) gene on chromosome 16p13 due to cytogenetic abnormalities [21]. The CBFB-MYH11 fusion reported with frequency of 10% in AML patients, also has been described in ME-1 cell line with eosinophilic abnormalities. CBFB-MYH11 fusion reported across all age categories, but mostly in young ages of 35 years [9]. In this study, the CBFB-MYH11 fusion gene detected with nested PCR by linking the primers as following: CBFB-A\MYH11-B1\MYH11-B2 for the first thermocycle run and CBFB\MYH11-D1\ MYH11-D2 for the second thermocycle run, positive result detected in four AML patients representing a frequency of 5.71% (Figure 2), agarose gel electrophoresis detected positive results with product size number of about 500 pb (Figure 3).

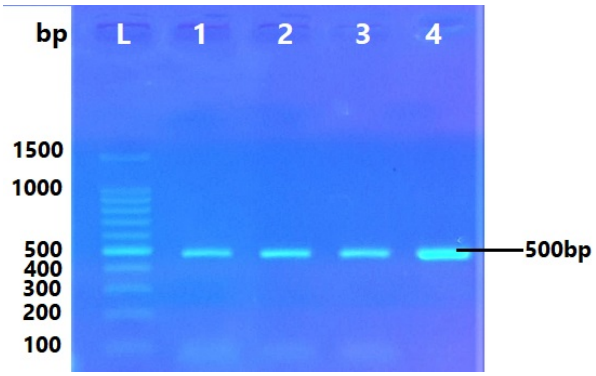

Fig 3. Agarose gel showing positive results detected with nested PCR for CBFB-MYH11 fusion gene. (L): ladder & lane 1-5: positive samples for the CBFB-MYH11 fusion (product size 500bp).

Same results were suggested by Biernacki et al and Huang et al, also studies by van Dongen et al suggested a frequency of 10% AML in ME-1 cell line, Although. Though most of these leukemias (50% or more) correspond to AML-M4Eo, CBFB-MYH11 transcripts have been found in a number of other AML types, including M4 without eosinophilic abnormalities, M2, M5, and, less often, M1, M6, and M7 [10,13,15].

Flow cytometry analysis

The results were checked using flow cytometry, which investigated the fusion genes depending on immunophenotype expression of the CD markers targeted for each fusion under study. The positive expression of the immunophenotype CD8 and CD2 was detected for PML-RARA and CBFB-MYH11, respectively. The positive results were determined by counting cells, and if they were above 20%, they were considered positive.

The negative control had less than 20% and was considered negative. Figure 4 shows the results.

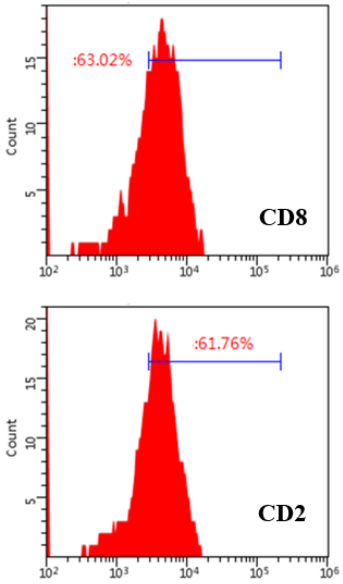

Fig 4. Flow cytometry immunophenotype expression showing positive results indicated higher than 20% for all types of conjugated antibodies both in a histogram and dot blot graphs.

In this study we analyzed the distribution of hematopoietic progenitors (myeloid progenitors) in peripheral blood of AML cells with PML-RARA and CBFB-MYH11. The results obtained were consistent with previous study by Dekking et al they analyzed the distribution of hematopoietic progenitors in bone marrow and reported that leukemic cells with CBFB-MYH11 fusion were positive for CD222, furthermore, in a similar study they tested the PML-RARA immunobeads assay on cell lines and a number of AML (APL subtype) patients samples, they demonstrated that CD8 works as a detecting antibody for the PE-conjugated anti-PML, and it tested positive for leukemic cells with PML-RARA fusion [23].

CONCLUSIONS

In conclusion, this study determined the frequency of PML-RARA and CBFB-MYH11 fusion genes in Iraqi AML patients based on previous research, expanding our understanding of the types of fusions present in AML and potentially guiding therapeutic strategies. The detection method used in this study could be considered a novel approach to diagnosing leukemia, offering a reliable, accessible, and cost-effective diagnostic tool that could provide more detailed diagnoses for patients.

CONFLICT OF INTEREST

None.

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