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Detection of DEK-NUP214 and BCR-JAK2 fusions in acute myeloid leukemia using nested PCR and immunophenotyping assay

Rafal F. J. Aleiqabie[1], Fahrul Huyop[2], Ahmed D. Jabbar[3]

[1] Department of Pathological analysis, College of Science, University of Wasit, Iraq
[2] Department of Biosciences, Faculty of Science, University Technology Malaysia, UTM Johor Bahru, Malaysia
[3] Department of Biology, College of Science, University of Wasit, Iraq

ABSTRACT

Leukemia development is intricately linked to genetic factors, which contribute to genomic instability and rearrangements. In hematological malignancies, especially AML, fusion genes, which result from the fusion of two distinct genes, are commonly observed. Among these, the BCR-JAK2 fusion gene is a distinctive feature of AML, while the DEK NUP214 gene is known to enhance protein synthesis and disease progression. However, the precise roles played by these fusion proteins remain elusive. This study aimed to use advanced techniques, including nested Polymerase Chain Reaction (PCR) and flow cytometry, to identify DEK-NUP214 and BCR-JAK2 fusion genes in a cohort of Iraqi individuals diagnosed with Acute Myeloid Leukemia (AML). The investigation revealed the frequency of the DEK-NUP214 fusion gene as 2.85%, while the frequency of the BCR-JAK2 fusion was 1.42%. Additionally, the immunophenotyping assay yielded varying expression levels of CD13, CD33, CD10, and CD19, ranging from moderate to low. Importantly, it successfully detected fusion genes in all AML samples that tested positive for these genetic alterations. Remarkably, cell count analysis demonstrated that over 20% of cells in positive samples expressed the targeted CD markers. This research sheds light on the prevalence of DEK-NUP214 and BCR-JAK2 fusion genes among AML patients in Iraq, enhancing our comprehension of fusion events and potentially influencing treatment strategies. The detection methodology presented in this study offers a dependable, accessible, and cost-effective diagnostic tool.

Keywords: acute myeloid leukaemia, fusion gene, DEK-NUP214, BCR-JAK2

Address for correspondence:

Rafal F. J. Aleiqabie,

Department of Pathological analysis, College of Science, University of Wasit, Iraq

Email: rafelfiras@gmail.com

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INTRODUCTION

The influence of genetics is significant in the development of leukemia. Cancer arises as a result of genomic instability and rearrangement, which encompass various alterations including as translocation, amplification, deletion, and inversion [1, 2]. Various factors contribute to the occurrence of DNA transcription errors, resulting in the formation of fusion genes, also known as chimera genes. These fusion genes are created by the combination of two distinct genes [3]. According to prior research, fusion genes are primarily observed in hematological malignancies [4]. Therefore, the presence of fusion genes was identified in cases of Acute Myeloid Leukemia (AML) as reported by [5]. The study conducted by He et al. (2016) identified the presence of the BCR-JAK2 fusion gene in a case of Acute Myeloid Leukemia (AML) that arose from a translocation event involving chromosomes 9 and 22 [6]. This translocation was found to be the only cytogenetic abnormality observed in the case. The BCR-JAK2 fusion gene has a shared breakpoint within the BCR gene, analogous to the breakpoint seen in the BCR/ABL p210 fusion gene [7]. The chimeric gene arises from a reciprocal translocation event involving chromosomes 9 and 22, leading to a double break on chromosome 9. This translocation event results in the formation of an in-frame fusion transcript known as BCR-JAK2 gene fusion, which occurs due to a rearrangement of the 22q11 and 9p24 regions [8, 9]. JAK2, a member of the Janus family of kinases, plays a crucial role in facilitating cytokine-mediated signalling [10]. The occurrence of gain of function mutations, which result in the amplification of cation and translocations involving the JAK2 gene, leading to subsequent activation of the JAK2 kinase, has been documented in different types of haematological neoplasms. Specifically, fusion events involving JAK2 have been observed with TEL/ETV6, PCM1, PAX5, RPN1, SEC31A, and the B-Cell Receptor (BCR) in patients diagnosed with T-cell leukaemia, acute myeloid leukaemia, Hodgkin lymphoma, and myeloproliferative neoplasms [10]. The Janus kinase 2 (JAK2) protein is a tyrosine kinase that does not possess receptor activity. It plays a crucial role in transmitting signals through various cytokine receptors and is necessary for the normal process of blood cell formation, known as haematopoiesis [11]. The activation of the JAK2-cytokine receptor complex results in the recruitment and subsequent phosphorylation of STAT5 proteins by JAK2. This phosphorylation event leads to the dimerization of STAT5 proteins and their subsequent translocation into the nucleus, where they promote the

transcription of target genes [12]. On the other hand, the DEK NUP214 gene, which arises from the chromosomal translocation (6:9), has been found to be correlated with approximately 1% of Acute Myeloid Leukaemia (AML) cases and myelodysplastic syndromes [13]. Provided evidence indicating that the DEK NUP214 gene promotes cell proliferation by enhancing the activity of mammalian Target of Rapamycin Complex 1 (mTORC1). Additionally, the authors demonstrated that the proliferative effect generated by DEK NUP214 can be counteracted by the administration of a mTORC1 inhibitor. Hence, it may be inferred that the mTOR inhibitor exhibits potential suitability for therapeutic intervention in individuals harbouring the DEK-NUP214 gene. The DEK-NUP214 gene arises from a rare fusion event involving exon 2 of DEK and exon 6 of NUP214, as reported [13]. The overexpression of DEK-NUP214 has been observed to enhance protein synthesis, cellular proliferation, and the onset of leukaemia. Nevertheless, the precise mechanisms by which these fusion proteins contribute to the development of leukaemia are not yet fully understood [14]. The expression of NUP214 is crucial for the controlled export of large molecules, and the occurrence of fusion genes containing NUP214 due to chromosomal translocation can potentially disrupt the nuclear-cytoplasmic transport system [15]. Hence, the main purpose of this investigation was to evaluate the prevalence of DEK-NUP214 and BCR-JAK2 fusion genes, which are frequently observed in Iraqi individuals diagnosed with acute myeloid leukaemia. This was accomplished by use nested Polymerase Chain Reaction (PCR) and flow cytometry methodologies.

METHODOLOGY

Sample collection

The current study was carried out in Wasit, Iraq, spanning from the 5th of May, 2021, to the 23rd of July, 2022. The study involved the collection of fresh blood samples from a cohort of individuals diagnosed with Acute Myeloid Leukemia (AML), specifically targeting patients within the age range of 25 years to 45 years. Blood samples from healthy individuals within the same age range were also collected as control specimens. A volume of 4 ml of freshly obtained blood was collected from both patients diagnosed with Acute Myeloid Leukemia (AML) and healthy individuals. The blood samples were collected using EDTA tubes and appropriately labeled. The materials obtained were subsequently distributed evenly across two 2 ml tubes containing EDTA of each sample. One tube was utilized for the purpose of RNA extraction, whereas the other tube was employed for conducting a flow cytometry experiment aimed at detecting CDs, hence facilitating the subsequent identification of the presence of fused proteins. All the aforementioned procedures were executed within a 24-hour timeframe in order to guarantee precise outcomes. The process of sample collection adhered to the ethical guidelines outlined in regulation 13398 of 2016, paragraph 13, as mandated by the Iraqi Ministry of Health. This regulation mandates that oral and written consent must be obtained from patients, and that the collection process must be conducted under the direct observation of medical staff.

Fusion gene detection

The frequencies of the DEK-NUP214 and BCR-JAK2 fusions were examined in a cohort of Acute Myeloid Leukemia (AML) patients from Iraq. RNA was isolated from blood samples and subsequently reverse transcribed into complementary DNA (cDNA) using an applied biosystem kit. The cDNA samples were utilized as a template for the targeted primer of the fusion gene being investigated. The amplification of the samples was carried out using multiplex PCR, following the procedure outlined in the study conducted by [16]. The amplification process and specificity of DNA were assessed by the utilization of agarose gel electrophoresis. The obtained results were seen using a UV transilluminator set at a wavelength of 320 nm. In addition, the study employed flow cytometry as a tool to examine fusion genes at the protein level. This method involved the use of conjugated antibodies, namely CD10, CD13, CD19, and CD33, to detect the targeted fusion events.

Statistical analysis

Statistical analysis of the research findings was conducted using GraphPad Prism version 9.4.1. The analysis encompassed the computation of the proportion of affirmative outcomes for the control and Acute Myeloid Leukemia (AML) patient samples acquired using agarose gel electrophoresis. Gel electrophoresis was employed as a means of detecting the fusion gene through the utilization of nested PCR. Furthermore, the outcomes of the flow cytometry test were evaluated by transforming the signals into digital format utilizing specialized computer software specifically created for the flow cytometry instrument.

RESULTS AND DISCUSSION

DEK-NUP214 fusion gene

The DEK-NUP214 fusion gene is regarded as a very uncommon kind of gene fusions in adult patients with Acute Myeloid Leukaemia (AML). However, it has been documented to occur in young AML patients who are 30 years old or younger [17]. The DEK-NUP214 fusion gene arises from the fusion of the DEK gene, a proto-oncogene located on chromosome 6, with the NUP214 gene, encoding nucleoporin 214, located on chromosome 9. This fusion gene has been observed in approximately 0.5%-4% of patients diagnosed with Acute Myeloid Leukaemia (AML), particularly in those with M2 and M4 subtypes of AML [18]. According to Saito et al. (2016), the fusion's encoded protein has been documented to function as a suppressor of cellular death and differentiation. The presence of the DEK-NUP214 fusion gene was identified by the utilization of Polymerase Chain Reaction (PCR), wherein the DEK primer was bound to the NUP214 primer. The findings were demonstrated using agarose gel electrophoresis, revealing PCR product of around 430 base pairs (Figure 1).

The frequency percentage for the targeted fusions in this investigation is depicted in figure 2. The presence of the DEK-NUP214 fusion was observed in two instances of Acute Myeloid Leukemia (AML), indicating a frequency of 2.85%.

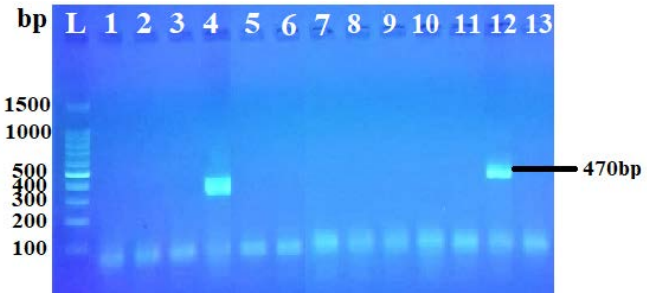

Fig. 1. Agarose gel electrophoresis of the DEK-NUP214 fusion gene detected by nested PCR. L, DNA ladder; lane 4 and 12 positive result samples for DEK-NUP214 fusion (product size 470 bp)

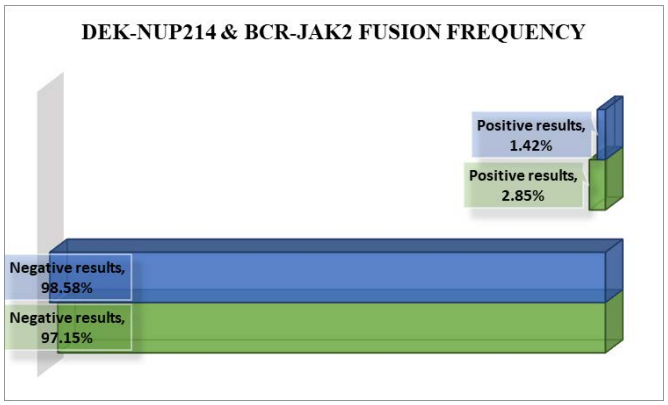

Fig. 2. Clustered bar chart showing frequency percentage of DEK-NUP214 and BCR-JAK2 fusion genes

The findings of a study conducted by Yang et al. (2020) corroborate the results obtained in this study. Yang et al. examined a case report of a 26-year-old patient with Acute Myeloid Leukemia (AML) and confirmed the presence of the DEK-Nup214 fusion gene using nested PCR and fluorescence in situ hybridization. Their results provide evidence that this fusion gene is more commonly observed in young AML patients, with a prevalence of 71% compared to adult patients. This disparity may be attributed to the fact that the presence of this fusion gene, as determined through an analysis of bone marrow morphology, is frequently accompanied by an elevated level of basophils, a rare occurrence.

BCR-JAK2 fusion gene

The BCR-JAK2 fusion gene is formed as a result of the fusion between the BCR gene, which encodes the breakpoint cluster region protein, and the JAK2 gene, which encodes the Janus kinase 2 protein. This fusion gene arises via a reciprocal translocation event between chromosome 9 and chromosome 22 [7]. The occurrence of this fusion is more prevalent in cases of Chronic Myeloid Leukaemia (CML) due to the presence of the same breakpoint in BCR as observed in CML. However, previous investigations have indicated a higher detection rate of this fusion in lymphoid/myeloid neoplasms [6]. According to Cuesta-Domínguez et al. (2012), it is widely thought that this fusion possesses a transformative capability, leading to the dysregulation of growth factor-dependent cellular proliferation and survival. The presence of the BCR-JAK2 fusion gene was identified using Polymerase Chain Reaction (PCR) in a single patient diagnosed with Acute Myeloid Leukaemia (AML). This was achieved by utilizing the BCR-F primer in conjunction with the JAK2-R primer. The outcome of this experiment was visualized through agarose gel electrophoresis, revealing a product of approximately 290 base pairs (Figure 3). BCR-JAK2 fusion was discovered in just one AML case, representing a frequency of 1.42% (Figure 3).

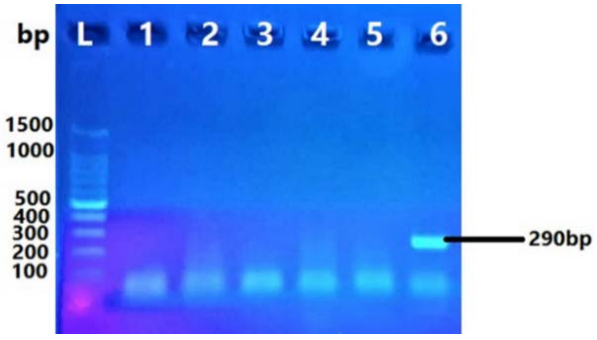

Fig. 3. Agarose gel electrophoresis of the BCR-JAK2 fusion gene detected by nested PCR. L, DNA ladder; lane 6, positive result samples for BCR-JAK2 fusion (product size 290bp); lane 1-5, negative results; bp, base pairs

The findings of He et al. (2016) support the notion that similar outcomes were observed. In their investigation, the authors examined a case report of acute myeloid leukemia (AML) and provided a comprehensive analysis of the BCR-JAK2 fusion gene. Their study confirmed that translocations are generally responsible for the emergence of fusion genes and also documented the potential of JAK2 to form fusions with transforming activity. According to a recent study conducted by Snider S. Jessica et al. (2020), it has been found that JAK2 is a genetic alteration that is frequently observed in myeloid and lymphoid malignancies [19, 20]. This alteration has been observed to fuse with several partners, with the BCR gene being one of the most prevalent partners.

Flow cytometry analysis

The findings of Fusions identification by nested PCR were verified and validated using a flow cytometry assay. This assay examined the fusion genes by analysing the expression of CD markers associated with the immunophenotype specific to each fusion being evaluated. This study examines the distribution of hematopoietic progenitors, specifically myeloid progenitors, in the peripheral blood of Acute Myeloid Leukaemia (AML) cells. The analysis focuses on the presence of targeted fusion events by assessing the surface marker expression of these leukemic cells.
The immunophenotype expression of CD13, CD33, CD10, and CD19 exhibited variability ranging from medium to low levels. However, it consistently detected positive results for all Acute Myeloid Leukaemia (AML) samples that were positively identi-fied with a specific fusion gene. In general, the proportion of cells expressing the targeted CDs was greater than 20% in all positive samples, as depicted in figure 4. In contrast to the positive result samples, the control samples and the samples from AML patients without identified fusions exhibited a negative result, denoted by a cell count of less than 20% for all conjugated antibodies target-ing CDs, as depicted in figure 4.

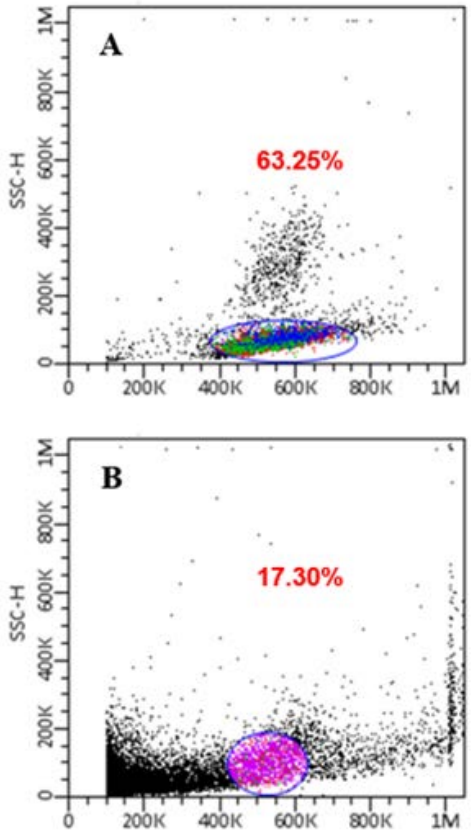

Fig. 4. Dot blot graph of flow cytometry immunophenotype expression showing positive and negative results, where (A) represent positive results indicated higher than 20% for this study targeted conjugated antibodies, while, (B) represent negative results indicated lower than 20% for this study targeted conjugated antibodies

The findings reported in the study conducted by Wang et al. (2022) demonstrate that the expression of DEK-NUP214 was assessed using flow cytometry in a cohort of 23 patients diagnosed with Acute Myeloid Leukemia (AML). The analysis revealed positive expression of CD13 and CD33 in these patients. The ob-tained results of the BCR-JAK2 flow cytometry assay exhibit sim-ilarities to the findings reported by Snider et al. (2020). In their study, Snider et al. investigated myeloid blasts in the bone marrow to identify the presence of BCR-JAK2 fusion. The flow cytometry results revealed a positive expression of CD10 and CD19.

CONCLUSION

In conclusion, this research has ascertained the prevalence of DEK-NUP214 and BCR-JAK2 fusion genes among AML patients in Iraq, building upon existing literature and enhancing our comprehension of the spectrum of fusion events observed in AML. Furthermore, these findings hold the potential to inform treatment approaches in the management of this disease. The detection methodology employed in this investigation can be regarded as an innovative strategy for the identification of leukemia, presenting a dependable, easily attainable, and economically effi-

cient diagnostic instrument that has the potential to furnish more comprehensive diagnoses for individuals.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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