

Detection of the E2A-PBX1 Fusion Gene in Acute Lymphoid Malignancies

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Abstract

Genomic instability is a hallmark of leukemia, often leading to chromosomal rearrangements and the formation of fusion genes such as E2A–PBX1, a well-characterized oncogenic transcription factor. This study investigated the prevalence of the E2A–PBX1 fusion gene among Iraqi patients with acute lymphoblastic leukemia (ALL) using nested PCR. A total of 100 blood samples from newly diagnosed ALL patients aged 5–25 years were analyzed alongside age-matched healthy controls. The fusion gene was detected in 5.3% of patients under 10 years and 3.1% of those aged 11–25 years, consistent with global reports. Agarose gel electrophoresis confirmed the fusion transcript by a distinct 280 bp PCR product. These findings highlight the diagnostic and prognostic significance of E2A–PBX1, particularly in pediatric and pre-B-cell ALL, and support its use as a molecular marker and potential therapeutic target.

Keywords: E2A -PBX1, fusion -genes, acute lymphoid leukemia, malignancies.

1. Introduction

Genetics plays a facilitative, as opposed to deterministic, role in the origin of leukemia. Genomic instability is the basic feature of oncogenesis where massive reconfigurations of the genome including

multiple translocations, amplifications, deletions, and inversions occur [1, 2]. The culmination of this perturbation is the formation of fusion genes also known as chimeric genes [3]. Several processes cause the distortion of DNA in the process of transcription, and the presence of two or

more genes in juxtaposition may lead to a hybrid gene structure [4]. In the past, it has been demonstrated that fusion genes play a role in the pathogenesis of hemato-logic malignancies [5]. Its t(1;19)(q23;p13) translocation was first reported in 1984 in a fraction of cases of pre-B-ALL in a number of studies, and followed up literature reviews indicate that the rearrangement is balanced or unbalanced, that is, a derivative chromosome 19 t(1;19) with the deletion of chromosome 19 but retention of 2 normal chromosomes 1 [6]. About 5–6% of kids and 3% of adults with ALL have the t(1;19) translocation [7]. In both the pediatric and the adult population, this chromosomal change is strongly correlated with precursor B-cell acute lymphoblastic leukemia (pre-B-ALL) with cytoplasmic IgM expression, However, despite the infrequent cases recorded in pro-B-ALL, and the more prevalent type of ALL, which is about 1 per cent., there are isolated cases of T-cell acute lymphoblastic leukemia (T-ALL) and AML, as well (Jacoby et al., 2014). In most instances with the t(1;19) translocation, a characteristic type of the immunophenotype is evident with a homogenous expression of CD19, CD10 and CD9, and absence of CD34 and part or

complete absence of CD20 [9, 10, 11]. Besides, the t(1;19)(q23;q32) translocation has been associated with a range of high-risk clinical phenotypes, such as leukocytosis, high serum lactate dehydrogenase, and the CNS manifestations [12]. The objective of the research was to determine the occurrence of E2A -PBX1 fusion gene in Iraqi patients with ALL aged between 5 and 25 years of age using a nested polymerase chain reaction (PCR) method. and to examine its potential usefulness in the ALL context as it is a diagnostic and prognostic molecular biomarker.

2. Methodology

2.1. Collection of Samples

This research was conducted in Wasit, Iraq, from May 5, 2024, until July 20, 2025. A total of 100 fresh blood samples were taken from patients with acute lymphoblastic leukemia (ALL), which was recently diagnosed by the physician and aged from 5 to 25 years and divided into two subgroups of patients younger than 10 years and older 11 to 25 years. Control samples included blood of age-matched healthy individuals. For each

of the study participants, 2 mL of fresh peripheral blood was obtained in ethylene-diamine-tetra acetic acid (EDTA)-treated tubes, carefully labeled, and then used for RNA extraction. All samples were taken with informed permission and under the direct observation of certified medical professionals.

2.2 Detection of Fusion Gene

The frequencies of E2A-PBX1 fusions were examined in fifteen adult acute lymphoblastic leukemia (ALL) cases from Iraq by the detection of fusion transcripts. The complementary DNA (cDNA) was synthesized by reverse transcription from total RNA isolated from peripheral blood samples utilizing an Applied Biosystems kit. The resultant cDNA was then utilized as a template for polymerase chain reaction (PCR) amplification using primers designed specifically for the fusion gene of interest. Amplification was performed in a multiplex PCR configuration following the approach of van Dongen et al. [13]. Agarose gel electrophoresis was done after amplification to ascertain the specificity of the DNA products. Visualization of the

gels was done on a UV transilluminator (320 nm).

2.3 Data Statistical Analysis

In the case of the dataset generated by GraphPad Prism version of 9.4.1, the percentage of positive result is computed in the case of the control group, as well as samples obtained from patients with acute lymphoblastic leukemia (ALL). The fusion gene was identified by agarose gel electrophoresis following nested PCR amplification.

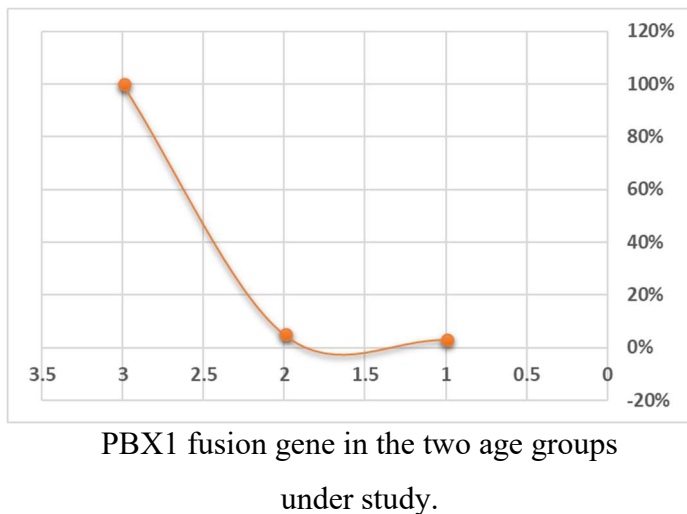
3. Results with Discussion

3.1 Fusion Gene E2A-PBX1

An example of oncogenic transcription factors produced due to chromosomal translocation is E2A PBX1 [14]. This fusion protein causes the aberrant transcriptional activation which leads to the endogenous differentiation pathway disruption and reorganization of signaling networks [14]. In terms of therapeutic relevance, E2A PBX1 expression is useful in identifying a unique subtype of ALL, is prognostically significant, as well as being a viable therapeutic target by direct transcriptomic

or proteomic inhibition and through modulation of its downstream signaling constituents [15]. In the current study, the E2A-PBX1 fusion was detected in about five percent of ALL cases in patients younger than ten years and in three percent of the group of patients aged eleven to twenty-five years (Figure 1), thus showing a higher prevalence in the pediatric subpopulation of the cohort studied.

Figure 1: A graph chart illustrating the examination of the frequency of the E2A-



The E2A-PBX1 fusion gene has been noted to occur between three and six percent in juvenile acute lymphoblastic leukemia (ALL) and is lower and less frequent in adult ALL at between two and four per cent. Notably, prevalence may be as high as 25 % in the pre-B ALL subtype that demonstrates a good level of

correlation with this type of immunophenotypic subtype [14, 16, 17]. Agarose gel electrophoresis was used to analyze the results and a PCR product of approximately 280 base pairs was identified (Figure 2).

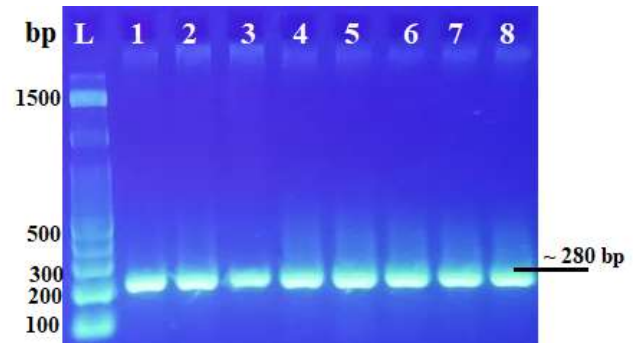


Figure 2: E2A-PBX Fusion showed detected by agarose gel electrophoresis, identified by nested PCR, demonstrated the presence of the target band in samples from individuals aged 10 years (lanes 1-5). In contrast, samples taken from patients aged 11-25 years also showed the E2A-PBX1 fusion band (lanes 6-8). The size of bands observed corresponded to the fragment size of around 280 bp. L: DNA ladder.

4. Conclusion

Extensively characterized as a potent oncogenic driver in acute lymphoblastic leukemia (ALL), the E2A-PBX1 fusion gene. Its pathogenic effect is

caused by a chromosomal translocation that causes aberrant transcriptional activation and leads to defects in cellular differentiation, and impacts on the regulation of the multiple signaling pathways. In the current study, this combination was observed in 5.3 percent of ALL cases among patients below the age of 10 years and a 3.1 percent in the age group of 11-25 years which represents the previously stated incidence of 3 - 6 percent in pediatrician ALL and 2 - 4 percent cases in adult cases. The good correlation that exists between this gene and pre-B immunophenotype is relative, and this genetic complication is most prevalent among younger children, in which the incidence can be as high as 25%, makes the E2A-PBX1 fusion of the greatest diagnostic and prognostic relevance. The investigation of the clinically relevant fusion transcripts was accomplished by polymerase chain reaction analysis followed by agarose gel electrophoresis; representative product obtained 280 base pairs. This methodological approach demonstrates the usefulness of the many molecular investigations to properly identify such fusions. All these results support the application of E2A-PBX1 to

illness classification using it as a molecular diagnostic marker and suggest that it is a potential biological target candidate to be used in future therapeutic procedures.

5. Future plans

Future studies should investigate E2A-PBX1 prevalence in larger ALL cohorts, using advanced molecular techniques to validate detection. Exploring associations with immunophenotype, treatment response, relapse, and survival may clarify its prognostic value. Additionally, investigating downstream signaling pathways could identify potential therapeutic targets and improve personalized treatment strategies for ALL patients

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