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Pattern of Translocations in Acute Leukaemia: A Single Centre Experience

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ABSTRACT A multifaceted approach is the current modality to the diagnoses of acute leukaemia. Given the importance of knowing chromosomal aberrations for rapid diagnosis and better treatment selection, this study assessed the frequency of common chromosomal translocations among acute leukaemia patients. In this retrospective study, both conventional karyotype and RT-PCR detecting translocations (HemaVision®-28N) were utilised in 400 patients with acute leukaemia of all subtypes in King Abdulaziz University, Jeddah (2015-2020). The researchers identified 38 acute leukaemia cases associated with translocations out of 400 new cases (9.5%), 326 samples were negative (81.5%) and 36 resisted PCR (9%). The most frequent observed translocation is t(9;22) (q34;q11.2) P190 (representing 28.9% of the total positive calls), followed by t(15;17) (q24;q21) (PML-RARA) and t(8;21) AML-ETO, each of which represents 13 percent. The resulting acute leukaemia translocation frequencies are similar to that published in the literature both locally and globally.

INTRODUCTION

Chromosomal translocations have an important role in carcinogenesis through gene fusions causing about 20 percent of human cancers (Streb et al. 2023; Panagopoulos and Heim 2022). Several gene fusions, particularly leukaemia associated markers for are clinically useful for the minimal residual disease (MRD) follow up (Kruse et al. 2020; Heuser et al. 2021).

In the United States, 59,610 new leukaemia cases were identified in 2023. An estimated 20,380 (34%) were cases of AML, and 6,540 (11%) were due to ALL (American Cancer Society 2023). In 25-30 percent of AML cases, balanced chromosomal rearrangements, especially translocations, occur. It received considerable attention because of its importance in the recognition of leukemogenesis genes and their relationship to the treatment of patients (Mrózek and Bloomfield 2008).

It was a significant advance to recognise a specific chromosomal translocation to assist in both the diagnosis of acute leukaemia besides predicting the future behaviour of the disease. To understand how translocations contributed to development of acute leukaemia, much attention was paid by those in this field. The paradigm was that acute leukaemia required two genetic lesions that prevented the differentiation of hematopoietic progenitors and generally preleukemia formation, as well as later proliferation, which usually led to point mutation (Brown 2022).

Molecular cytogenetic analysis has many advantages, such as the quick and comprehensive detection of known target translocations, as compared with classic cytogenetic analyses. Small samples with low quality reverse transcriptase are best candidates for RT-PCR (Mrózek 2022; Sabath 2019).

While conventional cytogenetic studies remain a cornerstone of genetic testing, the most effective tool to detect genetic lesions that define disease has been developed in molecular-based technologies. RT-PCR in the routine diag-

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nostic environment detects most translocations when assessed at molecular level. RT-PCR identification of major translocations of leukaemia has advantages over conventional cytogenetics, including short turnaround, no cell division requirement, detecting translocations missing from conventional cytogenetics (cryptic translocations) and the subsequent MRD testing with sensitive markers (Pourrajab et al. 2020; Limsuwanachot et al. 2016).

Objectives

The study aimed to identify the frequency of the molecularly defined genetic abnormalities in acute leukaemia on a local level and compare the resulting numbers to national and global statistics.

MATERIAL AND METHODS

Study Design

This study was a retrospective analysis conducted at King Abdulaziz University in Jeddah, Saudi Arabia, between 2015 and 2020. The aim was to assess the frequency of common chromosomal translocations in patients diagnosed with acute leukaemia. Both conventional karyotype analysis and reverse transcriptase polymerase chain reaction (RT-PCR) were employed to detect translocations in the patient samples.

Patient Population

The study included a total of 400 patients diagnosed with acute leukaemia of all subtypes. The patients' samples and accompanying epidemiological data were obtained as part of routine clinical care at King Abdulaziz University.

Conventional Karyotype Analysis

Conventional karyotype analysis was performed on the patient samples. This technique involves the microscopic examination of metaphase chromosomes prepared from cultured cells. The karyotype analysis aims to identify chromosomal abnormalities, including translocations, by examining the banding patterns and structural changes in the chromosomes.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RT-PCR was used to detect chromosomal translocations at the molecular level. The HemaVision®-28N commercial kit from DNA Diagnostic A/S was employed for this purpose. The kit is designed to detect specific fusion transcripts associated with known chromosomal translocations. The testing procedures were validated in King Abdulaziz University Hospital's Molecular Diagnostics laboratory with a sensitivity of 10^{-3} to 10^{4} for clinical trials using published primers and PCR conditions.

RNA Extraction and Reverse Transcription

Total RNA was extracted from the patients' whole blood samples to be qualitatively tested by HemaVision®-28N. The RNA extraction procedure followed the manufacturer's instructions provided with the HemaVision®-28N kit. Reverse transcription was then performed, converting the extracted RNA into complementary DNA (cDNA). This step allows for the amplification and detection of fusion transcripts associated with chromosomal translocations. Multiplex nested polymerase chain reactions and agarose gel electrophoresis follow the reverse transcription of RNA to cDNA. As a final step, chromosomes, fusion gene breakpoints, and mRNA splice variants from fusion genes are detected.

PCR Amplification and Detection

Multiplex nested PCR was performed using the cDNA generated from the reverse transcription step. The PCR amplification reactions were carried out using primers specific to the targeted fusion transcripts associated with the common chromosomal translocations. The PCR conditions and cycling parameters were conducted according to the manufacturer's instructions.

Gel Electrophoresis

Following PCR amplification, agarose gel electrophoresis was performed to separate and visualise the PCR products. The amplified DNA fragments were loaded onto an agarose gel and subjected to electrophoresis. The gel was then

stained with a suitable DNA dye, and the resulting bands were visualised under UV light.

Data Analysis

The results obtained from the conventional karyotype analysis (Fig. 1) and RT-PCR were analysed to determine the frequency of chromosomal translocations in the patient samples. The presence of specific fusion transcripts associated with known translocations was considered as a positive result.

RESULTS

The quantitative data obtained from the study includes an average age of the enrolled patients, which was found to be 24 years. The age range of the patients varied from 1 to 76 years. A total of 400 peripheral blood samples were collected for analysis. Out of these, 326 samples showed negative results, indicating the absence of acute leukaemia. However, 36 samples demonstrated resistance to PCR, suggesting a potential presence of the disease. Additionally, 38 samples tested positive for various translocations associated with acute leukaemia. The distribution of positive samples showed that 20 were from male patients, while 18 were from females.

The qualitative data provides important information about the characteristics of the patients and the study setting. All patients included in the study were diagnosed with acute leukaemia of all subtypes. The study was conducted at a tertiary health care centre in Jeddah. The diagnosis of acute leukaemia was confirmed using both bone marrow aspiration and trephine biopsy. Flow cytometry was also employed to further confirm the diagnosis.

Of note, the 36 resistant samples showed limited or no amplification during PCR analysis. PCR resistance can arise due to various factors, including the presence of inhibitors in the sample that interfere with the PCR reaction, mutations or variations in the target DNA sequence that prevent effective primer binding and amplification, or the absence of the target sequence altogether. When samples demonstrate PCR resistance, it means that either the target DNA sequence is not present in the sample, or it is present but cannot be effectively amplified using the specific PCR conditions and primers employed. This can hinder the accurate detection and analysis of the target DNA in the sample.

The most frequent observed translocation is t(9;22) (q34;q11.2) P190 (representing 28.9% of the total positive calls), followed by t(15;17) (q24;q21) (PML-RARA) and t(8;21) AML-ETO, each of which represents 13 percent.

Table 1 provides a breakdown of the different translocations associated with specific leukemia diagnoses and their respective frequencies and percentages in the sample. The t(9;22) (q34;q11.2) translocation, also known as the Philadelphia chromosome, is associated with acute lymphoblastic leukemia (ALL) subtype with the P190 fusion gene. This type of leukemia accounts for 28.9 percent of the cases in the sample. The t(15;17) (q24;q21) translocation, resulting in the PML-RARA fusion gene, is indic-

Table 1: The frequency and percentage distribution of different types of leukaemia diagnosis based on specific chromosomal translocations

	Frequency	*Percent*	*Diagnosis*
t(9;22) (q34;q11.2) P190	11	28.9	ALL
t(15;17) (q24;q21) (PML-RARA)	5	13	AML (M3)
t(8;21) AML-ETO	5	13	AML (M2)
t(12;21) (p13;q22) (ETV6-RUNX1)	4	10.2	ALL
t(8;21) (q22;q22) (RUNX1-RUNX1T1)	3	7.7	AML (M4)
inv(16) (p13;q22) (CBFB-MYH11)	3	7.7	AML(M4)
t(11;19) (q23;p13.1) (KMT2A-ELL)	2	5.1	AML (M4/M5)
t(1;16) (q31;q21)	1	2.5	AML (M6)
t(17;19) (q22;p13) (TCF3-HLF)	1	2.5	ALL
t(X;11) (q13;q23) (KMT2A-FOXO4)	1	2.5	ALL
del1(p32) (STIL-TAL1)	1	2.5	ALL
t(1;19) (q23;p13) (TCF3-PBX1)	1	2.5	ALL
Total	38	100	

Result:
46,XY,t(9;22)(q34;q11.2)

Result:
46,XY,t(9;22)(q34;q11.2)

1
2
3
4
5
6
7
8
9
10
11
12
t(9;22)(q34;q11.2)
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X
Y
t(9;22)(q34;q11.2)

Fig. 1. Illustrated conventional karyotype results for the most frequent detected translocate

ative of acute myeloid leukemia (AML) subtype M3, also known as acute promyelocytic leukemia. It accounts for 13 percent of the cases. The t(8;21) translocation, known as AML-ETO, is found in 13 percent of the cases and is associated with AML subtype M2. The t(12;21) (p13;q22) translocation, resulting in the ETV6-RUNX1 fusion gene, is found in 10.2 percent of the cases and is associated with ALL. Other translocations mentioned in the table are t(8;21) (q22;q22) (RUNX1-RUNX1T1), inv(16) (p13;q22) (CBFB-MYH11), t(11;19) (q23;p13.1) (KMT2A-ELL), t(1;16) (q31;q21), t(17;19) (q22;p13) (TCF3-HLF), t(X;11) (q13;q23) (KMT2A-FOXO4), del1(p32) (STIL-TAL1), and t(1;19) (q23;p13) (TCF3-PBX1). Each of these translocations accounts for 2.5 percent of the cases.

DISCUSSION

The presented information highlights the prevalence and significance of recurrent chromosomal translocations in adult acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML). These translocations play a crucial role in the development and classification of these diseases, and their identification can have implications for treatment strategies.

The t(9;22) translocation, also known as the Philadelphia chromosome, is a well-known aberration in ALL. Molecular-level analysis has revealed that approximately one-third of all adult ALL cases worldwide carry this translocation (Fukutsuka et al. 2022). This finding is consistent with the results obtained in the current study, which detected t(9;22) as a major aberration in all in-house ALL cases.

The t(15;17) and t(8;21) translocations have also been identified as common cytogenetic abnormalities in AML cases (Döhner et al. 2022). In a study of 5,876 newly diagnosed AML adults more than 60 years of age, the most common cytogenetic abnormalities included t(15;17) (q24.1;q21.2) (13%), Trisomy 8 (10%), t(8;21) (q22; q22.1) (7%), t11q23.3 rearrangements (6%), and inv(16) (p13.1q22)/t(16;16)(p13.1;q22) (5%) (Grimwade et al. 2010). In concordance with the global incidence, the study showed the highest frequencies of AML cases were found to be associated with t(15;17) (q24;q21) (PML-RARA) and t(8;21) AML-ETO. On a national level, King Abdulaziz Medical City in Jeddah, a local institution, had reached the above results in parallel. Their researchers reported the most frequent chromosomal aberration in their AML cases is t (15; 17) (17.2%). The next most common were complex karyotype (13.8%) and t(8;21) (5.7%) (Alsobhi et al. 2019).

Translocations involving specific chromosomal regions result in the fusion of genes, leading to the production of oncofusion proteins (Salokas et al. 2023). In the case of AML, the most frequent rearrangements are t(8;21), t(15;17), inv(16), and 11q23/MLL, with frequencies ranging from 3 percent to 10 percent. These translocations give rise to the AML1-ETO, PML-RARA, CBFB-MYH11, and MLL oncofusion proteins, respectively (Foucar and Anastasi 2015). It is noteworthy that t(15;17) is responsible for approximately 95 percent of acute promyelocytic leukaemia cases, which can be effectively treated with all-trans retinoic acid (ATRA) in its early phase (Thomas 2019).

The findings reported in the study provide important insights into the prevalence and clinical significance of specific chromosomal translocations in adult ALL and AML. The high frequency of t(9;22) in adult ALL cases worldwide underscores its importance as a diagnostic marker and potential target for therapeutic interventions. Similarly, the identification of the most common cytogenetic abnormalities in AML cases, such as t(15;17) and t(8;21), reinforces their clinical relevance in disease classification and treatment decisions. The consistency of these findings across different studies, including at a national level, adds to their credibility and generalisability. Overall, these observations contribute to the understanding of the molecular basis of leukaemia and may aid in the development of targeted therapies tailored to specific chromosomal aberrations.

CONCLUSION

Molecularly defined genetic abnormalities of acute leukaemia have a major effect on patients' treatment and prognosis. ALL with t(9;22) was the most frequently observed translocation associated with leukaemia in this study. The second in frequencies were t(15;17) (q24;q21) (PML-RARA) and t(8;21) AML-ETO. This study and similar studies are pivotal in selecting chemotherapy treatment and the choice of targeted

therapy like Tyrosin Kinase Inhibitors as well as a bridge for stem cell transplant.

RECOMMENDATIONS

Based on the findings highlighted in the manuscript, several recommendations can be made to treating physicians and researchers. Firstly, it is crucial for physicians to prioritise genetic testing in patients with acute leukaemia to identify molecularly defined genetic abnormalities. This information will aid in tailoring treatment plans and predicting prognosis. Incorporating genetic testing as a routine diagnostic tool can provide valuable insights for personalised care. Physicians should also consider the presence of specific genetic abnormalities, such as t(9;22), t(15;17) (q24;q21) (PML-RARA), and t(8;21) AML-ETO, when selecting treatment options. These findings suggest a need for targeted therapies like Tyrosine Kinase Inhibitors and specific chemotherapy regimens that have shown efficacy in addressing these genetic aberrations. Furthermore, researchers should continue to investigate the molecular basis of acute leukaemia and explore new therapeutic approaches targeting specific genetic abnormalities. Collaborative efforts between physicians and researchers can facilitate the development of novel treatment strategies and improve patient outcomes. For patients with high-risk molecular abnormalities, such as t(9;22), stem cell transplantation may be considered as part of the treatment plan. Close collaboration between haematologists and transplant specialists is essential to assess the eligibility and timing of transplant procedures. Finally, long-term follow-up of patients with acute leukaemia is crucial. Monitoring for relapse, evaluating treatment efficacy, and assessing the emergence of new genetic changes can guide subsequent treatment decisions and contribute to ongoing research efforts. By implementing these recommendations, treating physicians and researchers can optimise treatment strategies, improve patient outcomes, and contribute to the advancement of knowledge in the field of acute leukaemia.

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