Arch AHS

ORIGINAL ARTICLE

Archives of Allied Health Sciences 2024; 36(2): 44-58.

Cytogenetic Abnormalities in Hematolymphoid Tumors: Insight from Karyotyping of Bone Marrow Specimens in Northeast Thailand

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KEYWORDS
Cytogenetic abnormalities; Hematolymphoid tumors; Karyotyping; Bone marrow specimens.

ABSTRACT

Hematolymphoid tumors are classified into 1) lymphoid and 2) myeloid and histiocytic/dendritic neoplasms and are commonly caused by chromosomal abnormalities, such as chromosomal translocations. Previous studies reveal that genetic landscapes in acute myeloid leukemia (AML) vary between national populations. Therefore, this study aims to investigate the frequency of cytogenetic abnormalities using the conventional karyotype technique among hematolymphoid tumor patients in Northeast Thailand. Our finding of 314 hematolymphoid tumor patients demonstrates that the most common finding is chronic myeloid leukemia (CML) (187 cases, 59.55%), while AML was observed in 53 cases (16.88%). The third most prevalent finding is acute lymphoblastic leukemia (ALL), comprising 26 cases (8.28%). Other findings of hematolymphoid tumor patients in this region reveal myelodysplastic neoplasms (MDS) (19 cases, 6.05%) and lymphoma (9 cases, 2.87%). Among the 314 cases examined, the result of conventional karyotype shows most cases are normal chromosome (249 cases, 79.30%), while chromosome abnormality was seen in 26 cases (8.28%). Unfortunately, no metaphase or unsuccessful karyotype was revealed in 39 cases (12.42%). The most prevalent abnormality of 26 cases is the translocation between chromosomes 9q34 and 22q11.2, observed in 20.93% of cases and prominently associated with CML. Moreover, chromosome Y loss is demonstrated in both CML and AML (4 cases, 9.30%). Other chromosome aberrations are revealed in this study, including monosomy 21, marker chromosome (s), monosomy X, and trisomy 8. In conclusion, this study suggests that CML is the most common hematolymphoid tumor in the northeast Thai population, frequently associated with the translocation of chromosomes 9q34 and 22q11.2. This finding contributes to our knowledge about hematolymphoid tumors in specific regions in Thailand which might be useful for management system of hematolymphoid tumor diagnosis and treatment.

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Received: 18 March 2024/ Revised: 24 April 2024/ Accepted: 18 June 2024

Introduction

Hematolymphoid tumors originate from hematopoietic and lymphoid tissues, affecting the blood, bone marrow, lymph nodes, and the lymphatic system[1]. Consequently, these tumors can cause aplasia, myeloproliferative, and lymphoproliferation, including leukemias and lymphomas[2,3]. Unlike solid tumors, a common cause of these diseases is chromosomal abnormalities, such as chromosomal translocations[4,5]. Two groups of hematolymphoid tumors are classified by the 5th edition of the WHO classification as lymphoid and myeloid and histiocytic/dendritic neoplasms. Lymphoid neoplasms are categorized into three subgroups: B-cell lymphoid proliferation and lymphomas such as acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL), T-cell and NK-cell lymphoid proliferations and lymphomas, and stroma-derived neoplasms of lymphoid tissues. Similarly, myeloid and histiocytic/dendritic neoplasms are classified into nine subgroups as follows; (A) myeloproliferative neoplasms (MPN) including chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia, primary myelofibrosis, (B) myelodysplastic neoplasms (MDS), (C) myelodysplastic/myeloproliferative neoplasms (MDS/MPN) such as chronic myelomonocytic leukemia (CMML), (D) acute myeloid leukemia (AML), (E) secondary myeloid neoplasms, (F) myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK), (G) acute leukemias of mixed or ambiguous lineage, (H) histiocytic/dendritic cell neoplasms, (I) genetic tumor syndromes with predisposition to myeloid neoplasia[6,7].

A different approach to diagnosing and treating hematolymphoid tumors is typically used. Diagnosis is usually based on complete blood counts, bone marrow examination, and observation of symptoms[8]. Bone marrow biopsy should confirm the diagnosis and classification[6-9]. These tests are essential and must be conducted regularly to provide a prognosis that will influence the patient's choice of appropriate treatment. Additionally, cytogenetic testing is crucial for diagnosis, prognosis, and post-treatment monitoring[6-8]. Deletion or duplication, which may occur in part or the entire chromosome, has been implicated in developing many diseases.

Over the past 60 years, cytogenetic analysis of hematologic malignancies has significantly advanced, employing chromosome studies and molecular techniques to identify disease-specific abnormalities. With over 600 fusion genes and 1,000 balanced translocations discovered, these methods have become pivotal in diagnosing, treating, and prognosing hematologic cancers[10]. The molecular cytogenetic phenotype has led to tailored treatments, exemplified by successful gene-targeted therapy in CML[11]. There are many cytogenetic techniques, one of the most commonly used being the conventional karyotype[12,13]. This method can distinguish chromosome abnormalities by examining the number and structure. Cytogenetic abnormalities play a crucial role in the pathogenesis, providing more details on these disorders' prognosis, diagnosis, and clinical outcomes [6,7].

AML patients in Seattle, USA, with unsuccessful cytogenetics (UC) had a lower response to chemotherapy treatment and were associated with a poor prognosis[14]. In China, AML patients with cytogenetic abnormalities have shorter overall survival than patients with normal karyotypes[15]. In Thailand, 44.1% of MDS patients have chromosome abnormalities, with the most common abnormalities being monosomy 7 and trisomy 8, each detected in 26.7% of the patients. Furthermore, the age of Thai MDS patients is lower than that of the Western population. Moreover, the genetic landscapes of AML patients vary between national populations[16]. Recently, the report of chromosomal abnormalities in MDS patients in upper Northern Thailand showed that the frequency and pattern differed from other populations. In addition, the percentage of blasts in bone marrow in MDS patients with abnormalities of chromosome

7 and other complex chromosomes has a higher risk of progressing to AML[17]. With its unique demographic and environmental factors, Northeast Thai population may exhibit distinctive cytogenetic abnormalities in hematologic malignancies. Therefore, this research seeks to fill the existing knowledge gap by investigating the spectrum and frequency of cytogenetic abnormalities among hematolymphoid tumor patients in this region of Thailand.

Materials and methods

Study design and participants

A retrospective analysis of the karyotype results was conducted on bone marrow specimens obtained from patients sent to the Cytogenetic Laboratory, Department of Pathology, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, in 2020-2021. The standard of Cytogenetic Laboratory has been accredited by The Royal of Pathologists of Thailand. The specimens underwent conventional karyotyping techniques to detect chromosomal abnormalities. A total of 580 cases, comprising 298 cases in 2020 and 282 cases in 2021, were included in this study. The exclusion criteria for this study included 1) cases not diagnosed with hematolymphoid tumors or suspicious diagnoses and 2) cases with incomplete clinical data.

Demographic data, including age and gender, and clinical data, including the pathologist and internist diagnosis, were correlated with cytogenetic findings. This study performed descriptive statistical analyses. The Institutional Review Board of Khon Kaen University approved the ethical considerations for this study (HE661237).

Conventional karyotyping

The conventional karyotype analysis protocol outlined in this study involved a comprehensive process for cell culture, harvest, spread slide preparation, staining with Trypsin and Giemsa, and subsequent interpretation, as shown in the supplement data. Briefly, cells were initially cultured with RPMI1640 and fetal bovine serum for 24 hours. Methotrexate treatment was then introduced, followed by centrifugation, supernatant removal, and the addition of RPMI 1640. Thymidine treatment and subsequent incubation complete the cell culture process.

Harvesting involved colcemid treatment, potassium chloride (KCl) incubation, and fixation with methanol: acetic acid fixative. The cells underwent multiple centrifugation and fixative steps before being stored at 4 °C. The spread slide protocol included soaking cleaned slides in distilled water, gently resuspending harvested cells, applying samples to slides, drying on a heated platform, and labeling. Trypsin and Giemsa staining was followed in four Coplin jars, with trypsin treatment, phosphate buffer rinse, Giemsa dyeing, and dilute water washes.

The stained slides were then analyzed using light microscopy under 10X and 100X magnification. Two independent cytogeneticists conducted the analysis. Metaphase cell numbers were determined at 100X magnification and analyzed using Ikaros Karyotyping Software (MetaSystems, Altlussheim, Germany) and GenASIs Bandview software (Applied Spectral Imaging, California, USA). Interpretation using the International System for Human Cytogenomic Nomenclature System 2020 (ISCN 2020) was employed for result interpretation[18]

Results

The prevalence of hematolymphoid tumors in Srinagarind Hospital, Northeast Thailand

This study encompassed 580 cases, with stringent exclusion criteria to ensure a focused investigation. Cases lacking diagnoses of hematolymphoid tumors or presenting with suspicious diagnoses and those with incomplete clinical data were excluded from the analysis. Following these criteria, a robust dataset of 314 cases with confirmed hematolymphoid tumors emerged (Table 1). The gender distribution within

this subset revealed 153 cases (48.70%) among females aged 0 to 81 years. Males constituted 161 cases (51.30%), ranging from 2 to 89 years.

The five most prevalent diseases were identified among the hematolymphoid tumors investigated in this study (Table 1). CML emerged as the most common, constituting a substantial portion with 187 cases (59.55%). Following closely, AML was observed in 53 cases (16.88%) of the study cohort. ALL was the third most prevalent, comprising 26 cases (8.28%). MDS were identified in 19 cases 6.05%), and lymphoma cases totaled 9 (2.87%).

Table 1 The demographic data of 314 hematolymphoid tumor cases

Demographic data	Number of Cases (%)
Sex, cases (%)	
Male	161 (51.30%)
Female	153 (48.70%)
Median Age, years (range)	
0-50 years	159 (50.64%)
51-89 years	155 (49.36%)
Diagnosis, cases (%)	
- CML	187 (59.55%)
- AML	53 (16.88%)
- ALL	26 (8.28%)
- MDS	19 (6.05%)
- Lymphoma	9 (2.87%)
- Other*	20 (6.37%)
Total	314

Note: * Others, including ET for 6 cases, PCM for 5 cases, PV for 5 cases, PMF for 2 cases, CLL for 1 case, and CMML for 1 case.

Abbreviations: CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic neoplasms; ET, essential thrombocythemia; PCM, plasma cell (multiple) myeloma; PV, polycythemia vera; PMF, primary myelofibrosis; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia.

The prevalence of normal karyotype, no metaphase cells, and cytogenetic abnormalities in hematolymphoid tumors

The karyotype analysis yielded results across three distinct categories in the studied cohort. Among the 314 cases examined, 26 cases (8.28%) displayed abnormal karyotypes (cytogenetic abnormalities), with diagnoses further categorized into specific hematolymphoid disorders (Tables 2 and 3). Utilizing the International System for Human Cytogenomic Nomenclature 2020 (ISCN 2020) for accurate interpretation, the breakdown of abnormal karyotypes included 11 cases of CML, 9 cases of AML, 2 cases of ALL, 2 cases of lymphoma, and 1 case of PV and 1 case of primary myelofibrosis (PMF) (Table 2). Additionally, 39 cases (12.42%) exhibited no metaphase cells, and the majority, 249 cases (79.30%), displayed a normal karyotype.

Table 2 The prevalence of normal karyotype, no metaphase cells, and cytogenetic abnormalities in hematolymphoid tumors

Hematolymphoid tumors	Karyotype, cases (%)			Total cases (%)
	Normal chromosome	No metaphase cell	Abnormal chromosome	
CML	157 (83.96%)	19 (10.16%)	11 (5.88%)	187 (100%)
AML	33 (62.26%)	11 (20.75%)	9 (16.98%)	53 (100%)
ALL	19 (73.08%)	5 (19.23%)	2 (7.69%)	26 (100%)
MDS	17 (89.47%)	2 (10.53%)	0 (0.00%)	19 (100%)
Lymphoma	6 (66.67%)	1 (11.11%)	2 (22.22%)	9 (100%)
*Other	17 (85.00%)	1 (5.00%)	2 (10.00%)	20 (100%)
Total	249 (79.30%)	39 (12.42%)	26 (8.28%)	314 (100%)

Note: * Others, including ET for 6 cases, PCM for 5 cases, PV for 5 cases, PMF for 2 cases, CLL for 1 case, and CMML for 1 case.

Abbreviations: CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic neoplasms; ET, essential thrombocythemia; PCM, plasma cell (multiple) myeloma; PV, polycythemia vera; PMF, primary myelofibrosis; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia.

Table 3 The 26 cases of abnormal karyotyping were divided into diagnostic groups. The karyotype interpretation used the International System for Human Cytogenomic Nomenclature 2020 (ISCN 2020)

Diagnostic group	Karyotype (26 cases)	Sex	Age (year)
CML (11 cases)	46,XY,t(9;22)(q34;q11.2)[20]/46,XY[4]	Male	38
	46,XY,t(9;22)(q34;q11.2)[6]	Male	43
	46,XY,t(9;22)(q34;q11.2)[15]/46,XY[60]	Male	43
	46,XY,t(9;22)(q34;q11.2)[12]	Male	32
	46,XY,t(9;22)(q34;q11.2)[25]	Male	43
	46,XY,t(9;22)(q34;q11.2)[25]	Male	42
	46,XX,t(9;22)(q34;q11.2)[25]	Female	48
	46,XY,t(9;22)(q34;q11.2)[3]/46,XY[22]	Male	44
	46,XY,t(9;22)(q34;q11.2)[42]/46,XY[25]	Male	58
	45,X,-Y[9]/46,XY[17]	Male	52
	47,XY,+15[3]/46,X,-Y,+15[8]/46,XY[22]	Male	61

Table 3 The 26 cases of abnormal karyotyping were divided into diagnostic groups. The karyotype interpretation used the International System for Human Cytogenomic Nomenclature 2020 (ISCN 2020) (Cont.)

Diagnostic group	Karyotype (26 cases)	Sex	Age (year)
AML (9 cases)	46,X,-X,+mar[25]	Female	44
	47,XX,add(1)(p36.1),-6,-8,+mar1,+mar2,+mar3[5]	Female	0
	47,XX,+21[15]/46,XX[3]	Female	60
	48,XY,+8,+11[2]/46,XY[7]	Male	77
	46,XX,del(8)(q22),-21,+mar[25]	Female	12
	46,XX,add(15)(q26),-16,-21,+mar1,mar2[42]/46,XX[1]	Female	16
	47,XY,+4[11]/46,XY[28]	Male	66
	47,XX,+8[8]	Female	20
	45,X,-Y,t(8;21)(q22;q22.3)[10]	Male	5
ALL (2 cases)	46,XY,add(11)(q25)[25]	Male	14
	47,XX,+22[3]	Female	22
Lymphoma (2 cases)	51-68<3n>,XXX,+X,+1,+6,+18,+19,+19,+21,[17]	Female	69
	46,X,-X,-10,+mar1,+mar2[1]/46,X,-X,+18[1]/ 48,XX,del(1)(q25),+14,-18,-18,+mar1,+mar2,+mar3[1]	Female	40
PV (1 case)	46,XY,add(15)(q10)[15]	Male	58
PMF (1 case)	46,XX,t(11;12)(p15;q15)[11]	Female	68

Note: The number in [ ] represents the metaphase cell count for the karyotype. The "/" represents different clones of metaphase cells, also called mosaicism.

Abbreviations: p, short arm of chromosome; q, long arm of chromosome; t, translocation; add, additional material of unknown origin; del, deletion; mar, marker chromosome; 3n, near-triploidy; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; PV, polycythemia vera; PMF, primary myelofibrosis.

Recurrent rates of cytogenetic abnormality

The research findings on cytogenetic abnormalities in hematolymphoid tumors, encompassing 43 cases, reveal a diverse spectrum of recurrent patterns (Table 4). The most prevalent abnormality is the Philadelphia chromosome, resulting from the translocation between chromosomes 9q34 and 22q11.2, observed in 20.93% of cases and prominently associated with CML. For example, the karyotype of the 32-year-old male diagnosed with CML reveals 46,XY,t(9;22)(q34;q11.2)[12] as shown in figure 1A. In addition, marker chromosomes are identified in 9.30% of cases, indicating associations with AML and lymphoma. A karyotype of the 16-year-old female diagnosed with AML reveals 46,XX,add(15)(q26),-16,-21,+mar1,+mar2[42]/46,XX[1] indicating that 42 metaphase cells with additional chromosome material attached to 15q26, monosomy 16, monosomy 21, and 2 different marker chromosomes and 1 metaphase cell with normal female chromosome as figure 1B. Notable occurrences include the loss of the Y chromosome in males (6.98%), linked to CML and AML, and monosomy 21 (Figure 1B) and trisomy 8 (4.65% each) associated with AML (Figure 1F and 1G). Monosomy X in

females (4.65%) is also identified in AML and lymphoma cases (Figure 1C-1F). The mosaicism chromosome of the lymphoma patient with ISCN: 46,X,-10,+mar1,+mar2[1]/46,X,+18[1]/48,XX, del(1)(q25),+14,-18,-18,+mar1,+mar2,+mar3[1] was seen in 3 metaphase cells as figure 1C, 1D, 1E, respectively. Figure 1C demonstrates monosomy 10, monosomy X, and 2 different marker chromosomes (ISCN: 46,X,-10,+mar1,+mar2[1]). Additionally, the metaphase cell of Figure 1D indicates monosomy X and trisomy 18 (ISCN: 46,X,+18[1]). The metaphase cell of Figure 1E demonstrates deletion of 1q25, trisomy 14, nullisomy 18, and 3 marker chromosomes (ISCN: 48,XX,del(1)(q25),+14,-18,-18,+mar1, +mar2, +mar3[1]). The example of the 77-year-old male diagnosed with AML reveals a result of 48, XY,+8,+11[2]/46,XY[7] in 2 metaphase cells, while others are normal male chromosomes. This abnormality indicates trisomy 8 and trisomy 11 in 2 metaphase cells, as shown in figure 1F. The last sample with a representative of an abnormal karyotype is the 20-year-old female diagnosed with AML and reveals a result of 47,XX,+8[8], indicating trisomy 8 in 8 metaphase cells. Various other rare abnormalities, including additional material of unknown origin at chromosomes, a complex karyotype (near-triploidy), translocations, deletions, monosomies, and trisomies, are highlighted, contributing to a comprehensive understanding of the cytogenetic landscape in hematolymphoid tumors.

Table 4 Recurrent rates of 27 identified cytogenetic abnormality patterns and disease associations among 26 cases of abnormal karyotyping

Recurrent cytogenetic abnormality patterns	Recurrent rate case(s) (%)	Disease-associated
1. Translocation between chromosome 9q34 and 22q11.2 (Philadelphia chromosome)	9 (20.93)	CML
2. Marker chromosome (s)	4 (9.30)	AML, lymphoma
3. Loss Y in male	3 (6.98)	CML, AML
4. Monosomy 21	2 (4.65)	AML
5. Monosomy X in female	2 (4.65)	AML, lymphoma
6. Trisomy 8	2 (4.65)	AML
7. Additional material of unknown origin at chromosome 1p36.1	1 (2.33)	AML
8. Additional material of unknown origin at chromosome 11q25	1 (2.33)	ALL
9. Additional material of unknown origin at chromosome 15q10	1 (2.33)	PV
10. Additional material of unknown origin at chromosome 15q26	1 (2.33)	AML
11. Complex karyotype (Near-triploidy)	1 (2.33)	Lymphoma
12. Deletion of chromosome 1q25	1 (2.33)	Lymphoma
13. Deletion of chromosome 8q22	1 (2.33)	AML
14. Monosomy 6	1 (2.33)	AML
15. Monosomy 8	1 (2.33)	AML
16. Monosomy 10	1 (2.33)	Lymphoma
17. Monosomy 16	1 (2.33)	AML
18. Nullisomy 18	1 (2.33)	Lymphoma
19. Translocation between chromosome 8q22 and 21q22.3 (RUNX1/RUNX1T1 fusion gene)	1 (2.33)	AML
20. Translocation between chromosome 11p15 and 12q15	1 (2.33)	PMF
21. Trisomy 4	1 (2.33)	AML
22. Trisomy 11	1 (2.33)	AML
23. Trisomy 14	1 (2.33)	Lymphoma
24. Trisomy 15	1 (2.33)	CML
25. Trisomy 18	1 (2.33)	Lymphoma
26. Trisomy 21	1 (2.33)	AML
27. Trisomy 22	1 (2.33)	ALL
Total	43 (100%)	

Abbreviations: p, short arm of chromosome; q, long arm of chromosome; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; PV, polycythemia vera; PMF, primary myelofibrosis.

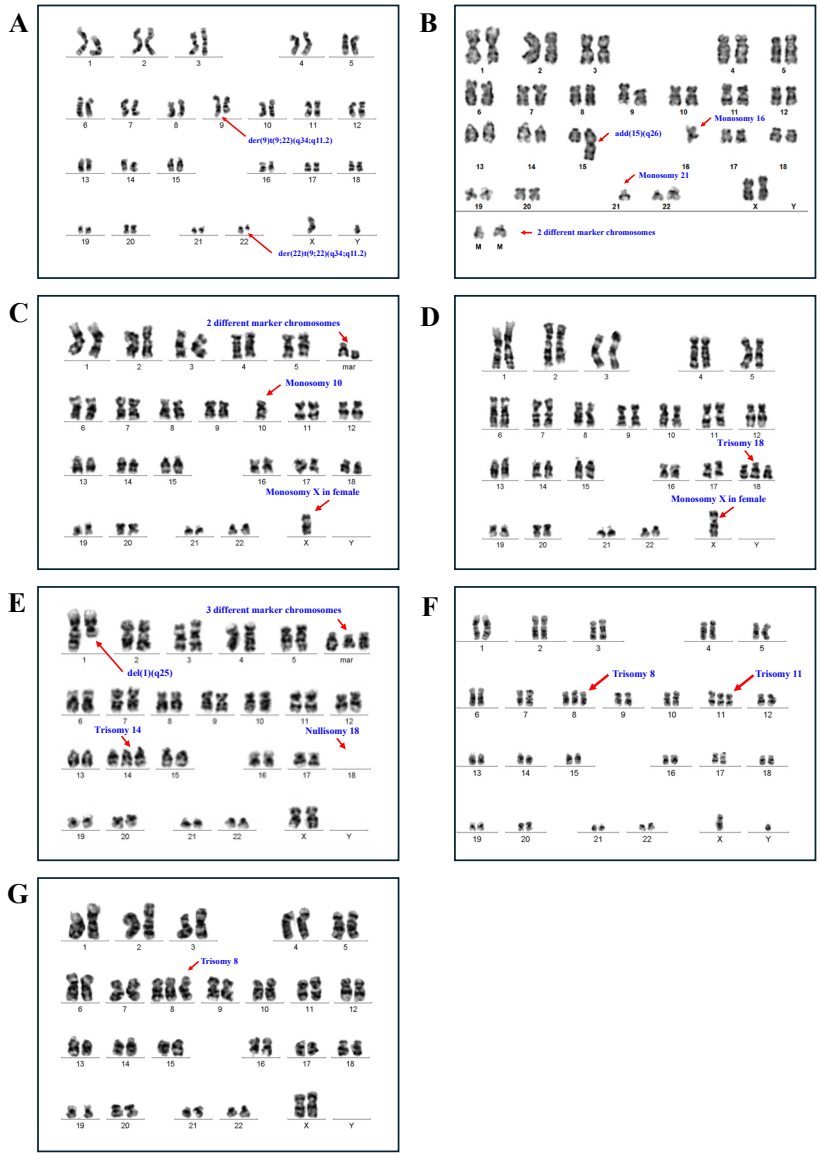

Figure 1 The representative karyotype of cytogenetic abnormalities in hematolymphoid tumors.
(A) The representative of CML reveals a result of translocation of 9q34 and 22q11.2.
(B) The additional chromosome material from an unknown origin was attached to 15q26, monosomy 16, monosomy 21, and 2 different marker chromosomes.
(C) This metaphase cell indicates monosomy 10, monosomy X, and 2 different marker chromosomes.
(D) Monosomy X and trisomy 18 was demonstrated in this metaphase cell.
(E) The deletion of 1q25, trisomy 14, nullisomy 18, and 3 marker chromosomes was represented.
(F) Trisomy 8 and trisomy 11 was showed.
(G) The karyotype revealed a result of trisomy 8.

Note: This karyotype analysis is conducted using GenASIs Bandview software (Figure 1B), and Ikaros Karyotyping software (Figure 1A, 1C-1F).

Abbreviations: add, additional chromosome material from an unknown origin; del, deletion; der, derivative chromosome; mar, marker chromosome; q, long arm of chromosome; t, translocation.

Discussion

The comprehensive investigation of 580 cases in this study, subject to stringent exclusion criteria, has provided valuable insights into the demographics and prevalence of hematolymphoid tumors. The robust dataset of 314 confirmed cases revealed a diverse spectrum of hematologic malignancies, with notable gender distribution and age ranges. Females accounted for 48.70% of the cases, from 0 to 81 years, while males constituted 51.30%, with ages ranging from 2 to 89 years. This indicates that hematolymphoid tumors exhibit heterogeneity in type and age, affecting both males and females globally[19].

Identifying the five most prevalent hematolymphoid tumors sheds light on the epidemiology of these diseases within the studied population. CML emerged as the most common, followed by AML, ALL, MDS, and lymphoma at 2.87%. The difference in incidence between our study and the previous one was observed. Our study found that the most common is CML, followed by AML, ALL, MDS, lymphoma, and other hematolymphoid tumors. On the contrary the survey from Zhang et al. found that the most common hematologic malignancies in 2019 were non-Hodgkin lymphoma, followed by other leukemia, PCM, ALL, AML, CML, and Hodgkin lymphoma. This may be based on the laboratory investigation decision made by the internist in our patients, which did not represent real disease incidents[19]. In addition, the number of newly diagnosed leukemia cases from 1990 to 2017 increased, especially AML and CLL, suggesting that it might become a major global public health in the future[20]. Similar to this study (Table 3), another study observed a higher incidence of CML in males compared to females. In terms of gender, the incidence and death of hematologic malignancies are generally higher in males than in females globally. This may be caused by hormonal, genetic, and environmental factors and requires further study[19].

Importantly, our study focused solely on karyotyping using bone marrow specimens, which may not precisely represent the incidence of hematolymphoid tumors. The karyotype analysis further enriched the understanding of hematolymphoid tumors by categorizing abnormal karyotypes into specific diagnostic groups. Among the 26 cases displaying cytogenetic abnormalities, CML and AML were predominant, reinforcing the association of these chromosomal aberrations with specific hematologic malignancies[8,21]. These findings underscore the heterogeneity of genetic alterations and their potential implications for disease classification and targeted therapeutic strategies in this patient population.

We differentiated the cases into diagnostic groups, leading to each treatment line according to the 27 recurrent cytogenetic abnormality profiles demonstrated among the 26 cases with cytogenetic abnormalities in this study. In our research, CML also became one of the hematolymphoid tumors with abnormal cytogenetic evaluation. The abnormally small chromosome, der(22)t(9;22)(q34;q11.2), also known as the Philadelphia chromosome, was the first recurrent cytogenetic abnormality found in CML as well as hematolymphoid tumors[12, 22]. Imatinib, a tyrosine kinase inhibitor (TKI), is the first targeted drug therapy for CML patients[11]. Some research presents an adverse prognostic outcome in TKI in specific adverse cytogenetic profiles, described as additional cytogenetic aberrations (ACA). The high-risk ACA group seems to suffer from worse prognostic outcomes compared to the low-risk group[21,23]. According to the management guidelines of CML patients in our practice, the clinical adaptation of the cytogenetic profile was still questionable for treatment choice and risk stratification. The cytogenetic profile could classify the risk group in the AML group to guide post-remission period treatment strategies. Our study demonstrated the cytogenetic abnormality indicating the intermediate and high-risk groups (complex karyotype and monosomal karyotype),

which decided the treatment line of allogeneic hematopoietic stem cell transplantation[24,25]. Complex karyotype in the context of AML is classified as ≥ 3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities, excluding hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities[8]. A monosomal karyotype is classified as the presence of two or more distinct monosomies (excluding loss of X or Y) or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding factor AML)[8]. Recurrent cytogenetic abnormalities were also identified in MPN, including PV and PMF. In our country's management guidelines, the cytogenetic feature was not directly involved in the management decision. However, the abnormality contributed to factors of risk stratification in some studies, including the "Three-tiered model Mutation-enhanced International Prognostic Scoring System for PV (MIPSS-PV)"[26]. In one case, our study also detected complex cytogenetic abnormalities in diffuse large B-cell lymphoma. None showed a loss of 17p, indicating a poor patient outcome[27]. A complex karyotype was observed, and the one in the bone marrow specimen strongly affected the prognostic outcome compared to those with a normal karyotype or single karyotypic abnormality[28]. Our study provides information on recurrent cytogenetic abnormality in the northeast Thai population, contributing valuable insights for ongoing and future research endeavors.

Additionally, our study's observation of cases with no metaphase cells and a majority with a normal karyotype highlights the variability in cytogenetic patterns within the studied population. As found in a previous study, the absence of metaphase cells for analysis can occur in hematologic malignancies with high counts[29]. These findings underscore the complexity of hematolymphoid malignancies and emphasize the need for further investigation.

Conclusion

This study thoroughly examined the details of hematolymphoid tumors in the northeast Thai population, covering their demographics, incidence rates, and genetic characteristics. The findings contribute significantly to our knowledge about these cancers, laying the groundwork for improved diagnostic methods and treatment approaches and guiding future research.

Take home messages

In this study of northeast Thailand, chronic myeloid leukemia (CML) emerged as the most prevalent hematolymphoid tumor. Conventional karyotype analysis revealed an 8.28% incidence of chromosome abnormalities, notably the Philadelphia chromosome translocation (9q34 and 22q11.2). These findings inform Thailand's health management strategies for hematolymphoid tumors.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This research was supported by the Cytogenetics Laboratory in the Division of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, to fulfill the patient information and samples. It was supported by the fund for invitation research (IN66086), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

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Supplementary

The standard protocol of karyotype in this study will be used as follows.

The process of cell culture

1. Grow cells with 5 mL of RPMI1640 (4:1 ratio of RPMI1640: fetal bovine serum), and incubation at 37 °C in a 5% CO_2 incubator for 24 hours.
2. Add 100 µL of methotrexate (MTX) and incubation at 37 °C in a 5% CO_2 incubator for 17 hours.
3. Centrifuge at 3,000 rpm for 5 min and drain supernatant; after that, add RPMI 1640 6 mL (Repeat steps 3, 2 times).
4. Add 0.01 M thymidine 100 µL and drain supernatant after that incubation for 5-6 hours at 37 °C in a 5% CO_2 incubator.

Harvest cell protocol

1. Add 1 mL of colcemid and incubate at 37 °C in a 5% CO_2 incubator for 15 min.
2. Centrifuge at 3000 rpm for 5 min and drain supernatant.
3. Add 10 mL of 0.075 M KCl and incubate in a water bath at 37 °C for 15 min.
4. Centrifuge at 3000 rpm for 5 min and remove supernatant.
5. Carefully add 6 mL of fresh fixative (3:1 ratio of methanol: acetic acid) to the cells while vertexing.
6. Centrifuge at 3000 rpm for 5 min and remove supernatant.
7. Add 6 mL of fresh fixative to the cells while vertexing (Repeat the step 5 & 6 for two times).
8. The cells are stored at 4 °C for repeating.

The protocol of spread slide

1. Take the cleaned slides and soak them in distilled water at room temperature.
2. Chromosome-harvested culture cells are gently resuspended with pasture pipettes, and a small number of cells are aspirated.
3. Apply 3-4 drops of samples to the slide at the end of the pasture pipette about 1 foot from the slide
4. Wipe the back of the slide dry and place the slide on a heating platform set to 60 °C.
5. Completely write the name and lab number on the slide head.
6. Leave the slides warm for 1 day, and then the slides are dyed.

Staining steps with Trypsin and Giemsa

Trypsin, a proteolytic enzyme, denatures euchromatic histones in DNA regions. Therefore, the regions will appear as bright bands when stained with Giemsa, whereas highly condensed chromatin with a large proportion of histones (protected from trypsin) will be dark bands.

The protocol is the following solutions to 4 Coplin jars.

1. The slides will be treated with trypsin for 6-7 seconds and dipped in the first Coplin jar, composted with 50 mL of phosphate buffer, for 5 seconds. Then, quickly rinse in the jar.
2. Then, leave each slide in the second jar for 8 minutes; this jar contains 50 mL of Giemsa dye (mixing 45 ml of phosphate buffer with Giemsa stock).

3. The third and fourth jars contain 50 mL of dilute water. The slides will be dipped in each jar for at least 30 seconds, and a quick rinse will allow them to dry.
4. Analyze cells with a light microscope under 10X and 100X magnification.

Interpretation

Two independent cytogeneticists collect the data on metaphase cell numbers to evaluate the quantity of chromosome analysis. Metaphase cell numbers are determined at 100X magnification and analyzed using Ikaros Karyotyping software (MetaSystems, Altlussheim, Germany) and GenASIs Bandview software (Applied Spectral Imaging, California, USA). Moreover, the result and interpretation of chromosomes are performed using the International System for Human Cytogenomic Nomenclature System (ISCN). The chromosome abnormality, either numerical or structural, is counted as abnormal.