Loss of Y Chromosome in Bone Marrow Cells with Hematologic Malignancies: A Retrospective Study

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ABSTRACT

Hematological diseases are characterized by changes such as chromosomal abnormalities, the activation of proto-oncogenes and inactivation of tumor suppressor genes in hematopoietic cells. The loss of chromosome Y (LOY) is frequent in the hematopoietic cells of older men. It has been accepted that LOY is related to the normal aging process for many years. However, some studies have shown that LOY in blood cells may be related to disease processes. We aimed to show whether LOY in patients with hematological malignancy is due to aging or a somatic chromosomal mutation seen in hematological malignancy. We conducted cytogenetic analysis on bone marrow samples obtained from 247 male patients between 2001 and 2021. Genetic test results for pre-diagnosed patients in the hematology department were conducted at the Genetic Diseases Assessment Center at Akdeniz University Faculty of Medicine. Patients are grouped into acute lymphoblastic leukemia (ALL) (n= 8), acute myeloid leukemia (AML) (n= 19), chronic lymphocytic leukemia (CLL) (n= 15), lymphoma (n= 49), myelodysplastic syndrome (MDS) (n= 54), multiple myeloma (MM) (n= 65) and myeloproliferative neoplasms (MPN) (n= 12). The 100% LOY was observed in 31,81% (n= 7) AML, 27.27% (n= 6) MM, 18,18% (n= 4) MDS, 9.09% (n= 3) ALL, 9,09% (n= 2) MPN, and 4.54% (n=1) lymphoma. The percentage of LOY in AML patients was significantly higher than that in lymphoma, CLL, MM, MDS patients, and control groups ($p< 0.01$). However, we found no statistically significant relationship between the percentage of LOY and advanced age in patients. Our data revealed that LOY was not associated with age, but rather with the disease, and it might be a chromosomal marker for AML. Further studies are needed to support our suggestion.

Keywords: Blood cells, Cytogenetics, Hematological malignancies, Sex chromosome

INTRODUCTION

Chromosomal abnormalities, including translocations, deletions, duplications, inversions, and aneuploidy, strongly impact most cancer types.[1] The mechanisms underlying many of these aberrations have been clearly explained.[1] Loss of sex chromosomes (LOS) has been reported in various cancers, including lung[2], pancreatic[3], colorectal[4], breast[5,6], and hematological cancers.[7] While the loss of Y chromosome (LOY) is detected in a wide range of hematological malignancies, it precise role in disease development remains unclear.[8]

In 1963, Jacobs et al. first described the loss of the Y (LOY) chromosome in cultured peripheral blood leukocytes[9], and they found a positive correlation with the formation of micronuclei containing ChrY.[10] This result demonstrates that during mitosis, the failure of correct segregation of ChrY is a considerable cause of LOY.[11] However, the pathological results of LOY have been inexplicable.[12]

LOY is the most common somatic genomic alteration observed in men's blood and bone marrow cells.[13]. In the literature, LOY is observed in the bone marrow cells of healthy elderly males and is considered normal age-related phenomenon.[14-17]

However, LOY is also observed in older males with hematological disease.[18] Our study aims to investigate whether the percentage of LOY in male bone marrow cells is age-dependent or simply serves as a cytogenetic marker for hematological malignancies.

PATIENTS AND METHODS

We designed this study as a retrospective study. Male participants had conventional chromosomal banding analysis results from derived from bone marrow cells at Akdeniz University Genetic Diseases and Assessment Center. All male patients were evaluated by hematologists at the Department of Hematology in the same university.

We enrolled 247 male patients, both with and without hematological malignancies. For inclusion in our study, we required that 247 males showed LOY in at least three cells. LOY was found in 223 patients with hematological malignancies, and 24 patients who were determined to have no hematologic malignancy by the hematologic department were included in our study as the control group. We evaluated conventional chromosomal banding analysis results derived from male patients' bone marrow cells. We categorized 223 male patients into five groups based on the percentage of LOY observed in every 20 cells counted: 13 patients with 5 to 10% of cells showing LOY, 64 patients with 11% to 24% of the LOY, 107 patients with 25 to 75% LOY, 17 patients with 76 to 99% of the LOY, and 22 patients with 100% LOY. In the control group, there were 7 male participants with 11 to 24% of cells showing LOY, 11 male participants with 25 to 75% of cells showing LOY, 4 male participants with 76 to 99% of cells showing LOY, and 2 male participants with 100% of cells showing LOY.

Statistical Analysis

First of all, the conformity of quantitative variables to normal distribution was tested with the Kolmogorov-Smirnov test. Then, Spearman correlation analysis was applied to examine the relationship between quantitative variables. Next, independent groups were compared with the Kruskal Wallis H test for quantitative variables that did not conform to normal distribution. Hence, Bonferroni-corrected Mann-Whitney U test was used to make multiple comparisons between groups according to Kruskal Wallis H test results. The descriptive statistics of the variables that did not fit the normal distribution were given as the median (25th-75th percentile). P values less than 0.05 were considered statistically significant.

This study received ethical approval from the Akdeniz University Clinical Research Ethics Committee (Approval number: KAEK-604; Date:August 18, 2021).

RESULTS

We conducted a search of 5000 reports on bone marrow cytogenetics from male patients spanning twenty years and identified a total of 247 males with LOY. We calculated the incidence of LOY to be 5% (247/5000). In the patient group, 66 patients with MM (30%, n= 66), 54 patients with MDS (24%, n= 54), 49 with lymphoma (22%, n= 49), 19 with AML (8%, n= 19), 15 with CLL (7%, n= 15), 12 with MPN (5%, n= 12) and 8 with ALL (4%, n= 8) were identified. Eight out of 223 patients had regular and isolated LOY in cytogenetic reports. Among them, four patients had multiple myeloma (MM), three had myelodysplastic syndrome (MDS), and one had acute myeloid leukemia (AML). Additionally, 11 patients with MM, three with ALL, two with MDS, two with AML, one with lymphoma, and one with CLL demonstrated a complex karyotype accompanied by LOY.

The proportion of LOY varied from 5% to 10% of cells in 5% of the total patient population, 11% to 24% of cells in 29% of the total patients, and 25% to 75% of cells in 48% (n= 107) of patients, with the greatest number of patients belonging to this group. There were 8% (n= 17) patients with a percentage of LOY, which was seen in 76% and 99% of cells. In addition, there were 10% (n= 22) patients with only 100% LOY. The distribution of the percentage of LOY and the hematological malignancies groups is given in Table 1 in detail.

In the control group, there are 29% (n= 7), 46% (n= 11), 17% (n= 4), and 8% (n= 2) males with 11

Table 1. The distribution of the percentage of LOY and the hematological malignancies groups

Percentage of LOY	Hematologic malignancies groups (number of patients)							Total (n=223)
	AML (n=19)	ALL (n=8)	CLL (n=15)	Lymphoma (n=49)	MDS (n=54)	MM (n= 6)	MPN (n=12)	
5-10%	8% (n=1)	8% (n=1)	0%	31% (n=4)	22% (n=22)	31% (n=31)	0% (n=0)	6% (n=13)
11-24%	5% (n=3)	0% (n=0)	8% (n=5)	23% (n=15)	23% (n=23)	36% (n=36)	5% (n=5)	29% (n=64)
25-75%	3% (n=3)	5% (n=5)	7% (n=8)	24% (n=26)	27% (n=27)	28% (n=28)	6% (n=6)	48% (n=107)
76-99%	29% (n=5)	0% (n=0)	11% (n=2)	18% (n=3)	18% (n=18)	18% (n=18)	6% (n=6)	7% (n=17)
100%	32% (n=7)	9% (n=2)	0%	5% (n=1)	18% (n=18)	27% (n=27)	6% (n=6)	10% (n=22)

to 24%, 25 to 75%, 76 to 99%, and 100% of cells showing LOY respectively.

LOY was observed in the patient group in both isolated and mosaic forms, with a rate of 71%, comprising the largest patient population. Secondary anomalies combined with LOY, complex karyotypes accompanied by LOY, regular isolated LOY, hypodiploidy with LOY, and hyperdiploidy with LOY were observed at rates of 8%, 8%, 4%, 4%, and 1% respectively.

It was determined that the percentage of LOY was not associated with age (r= 0.054; p= 0.396). Statistical findings showing whether the LOY changes significantly according to the clinical diagnosis were given in Table 2. The change in LOY according to diagnosis was statistically significant (p= 0.004). The results of the multiple comparison tests, conducted to investigate the source of significance, revealed the following findings: The percentage of LOY in AML patients was significantly compared to patients with lymphoma, CLL, MM, MDS, and control groups (p< 0.01). Furthermore, the percentage of LOY observed in patients diagnosed with ALL was significantly higher in comparison to lymphoma patients (p= 0.049).

DISCUSSION

LOY is detected as the sole cytogenetic anomaly in various hematologic malignancies in male bone marrow specimens. In many reports, the percentage of LOY is associated with various hematologic malignancies.[19-21] If there is an association between the percentage of LOY and hematological malignancy, it can be an indicator providing information about factors such as prognosis and remission in patients. As the incidence of MDS increases with age, it has been demonstrated that LOY leads to a good prognosis in patients with MDS.[22] In our study, a statistical analysis revealed a greater occurrence of LOY in AML patients compared to MDS patients. The results demonstrate a higher

Table 2. Descriptive statistics and comparison results on the percentage of LOY by groups

Groups	Percentage of LOY	X^2	p	Multiple Comparisons Tests
Control	32.5 (20-76.43)			
AML	88.67 (60-100)			
ALL	52.50 (33.08-92.86)	21.023	0.004^k	Lymphoma-ALL (p= 0.049)
CLL	28.57 (20-54.55)			Lymphoma-ALL (p< 0.001)
LYMPHOMA	28 (16.33-43.65)			CLL-AML (p= 0.007)
MDS	29.29 (20.63-61.25)			MM-AML (p< 0.001)
MM	34.17 (19.69-60)			MDS-AML (p= 0.001)
MPN	34.17 (23.97-83.33)			Control-AML (p= 0.008)

X^2: Kruskal Wallis H test test statistics; ^{k}= Kruskal Wallis H test
Descriptive statistics are given as the median (25th to 75th percentile).

percentage of LOY in AML patients. Additionally, the presence of pediatric individuals among AML patients led us to consider the possibility that the LOY may be associated with hematological malignancy rather than age. Our study evaluated the prognosis of MDS patients according to the Revised International Prognostic Scoring System (IPSS-R). While LOY was observed as a regular isolated anomaly in 6% (n= 3) and mosaic in 85% (n= 46) of the 54 MDS patients, other chromosomal abnormalities were found in conjunction with the LOY in 9% (n= 5). By IPSS-R, two of these five patients had a poor prognosis while the others had a very poor prognosis. Nomdedeu et al. demonstrated that regular isolated LOY was classified as a very good risk category according to IPSS-R for MDS.[23] Since two of the three patients with regular isolated LOY continued to be followed up at an external center, the IPSSR score of only one patient could be calculated. According to the IPSS-R, this patient's prognosis was assessed to be between very good and good.

In 2000, Wiktor, et al. published an article indicating that high LOY levels (> 75%) were associated with hematologic disease.[13] In our study, the majority of the patients (n= 107) had LOY values between 25% and 75%, while 39 patients had a high LOY (> 75%). Additionally, the percentage of LOY in AML patients was significantly higher than that in lymphoma, CLL, MM, MDS, and control groups ($p< 0.01$). Moreover, the percentage of LOY observed in patients diagnosed with ALL was significantly higher than that in lymphoma patients ($p= 0.049$).

Wiktor et al. reported a clinically significant association between the percentage of LOY in males over the age of 50 years diagnosed with MDS, MPN, and AML. They evaluated LOY in 75% or more metaphase cells, and a correlation was noted between the percentage of LOY, age, and hematologic disease.[24] However, in our study, although 209 patients were over 50 years old and 14 patients were younger than 50, we found the percentage of LOY was not associated with age ($r= 0.054$; $p= 0.396$).

Wong et al. demonstrated that 18 out of 38 patients (group 1) had 75% to 99% metaphase cells with LOY; only one patient was diagnosed with AML, while none were diagnosed with MDS in group 1.[25] In contrast, in our study, five patients were diagnosed with AML and three patients with MDS, all having LOY percentages ranging from 75% to 99% LOY. In their study, 9 out of 38 patients (group 2) with MDS had 100% metaphase cells with the LOY in the group 2.[25] During our study evaluation, it was found that seven participants had AML, while four patients had MDS, all exhibiting a 100% LOY in bone marrow metaphase cells. Patients with other diagnoses had 100% LOY, which were shown in Table 1. Wong, et al. determined that the mean age was 82 in patients with 100% LOY, but we found the mean age was 70 in patients with 100% LOY.[25]

Moreover, Wong, et al. reported that they found the incidence of LOY was 5% of 142 patients with hematological malignancies.[25] In our study, the prevalence of males with LOY was determined to be 5%. This finding is in agreement with previous literature.

Holmes et al. found that many AML patients with LOY at the time of diagnosis revert to a normal karyotype during the remission stage. Consistent with this study, in our research, two AML-M2 patients had t(8;21) with LOY at the time of diagnosis and were found two have a normal karyotype in the remission stage.[26] These patients were 12 and 57 years old, respectively. Our study results suggested that LOY may be associated with diseases rather than age. Further studies are needed to investigate the association between LOY and hematological malignancies.

Huh et al., analyzed 868 patients with hematologic diseases using bone marrow samples and identified a regular LOS in 5.1% of the study group.[27] However, none of them were diagnosed with ALL. In our study, we identified a regular LOY in 3.6% of the patients with hematologic malignancies, and we observed that there were no ALL patients with regular LOY. Nevertheless, Gupta et al. reported three cases with pediatric ALL and LOY in their bone marrow samples. Notably, in their study, the LOY co-occured with an additional Philadelphia (Ph) chromosome in the first case.[28] Secondary chromosomal aberrations in Ph+ ALL are known to be associated with a worse prognosis. Similarly, in our study, one out of eight ALL patients with LOY had Ph chromosomes. This finding represents the

second case of concomitant Ph positivity and LOY. This 18-year-old ALL patient had a karyotype of 45,X,-Y,t(9;22)(q34;q11),del(11)(q23),del(13)(q14)[3]. The detection of the Ph chromosome in the bone marrow sample and the poor prognosis of our patient is align with the results of Gupta et al. [28] Furthermore, the reversion of the patient's karyotype to normal before bone marrow transplantation provides additional evidence that LOY may be associated with hematological malignancies.

Finally, it is controversial whether LOY is a condition that develops with age or hematological malignancy. Further studies can shed light on our understanding of the phenomenon.

In conclusion, we found a statistically significant relationship between the percentage of LOY in AML patients as opposed to other hematologic malignancies. Furthermore, our findings suggested that LOY was not associated with age but rather with the specific disease. This underscores the potential significance of LOY as a chromosomal marker in hematologic malignancies or specific subtypes. Further research is required to determine the role of LOY in these circumstances.

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