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Research Article

Genetic Abnormalities Detected in Non-Hodgkin Lymphoma

Tuba Bulduk,[1] Eren Gunduz,[2] Beyhan Durak Aras,[3] Busra Emir,[4] Serap Isiksoy[5]

[1]Department of Hematology, Gulhane Training and Research Hospital, Istanbul, Turkey
[2]Department of Hematology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[3]Department of Genetics, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[4]Department of Biostatistics, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey
[5]Department of Pathology, Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey

Abstract

Objectives: To investigate the association between clinical features and fluorescence in situ hybridization (FISH) results of patients with non -Hodgkin lymphoma (NHL).

The advances in molecular biology, cytogenetics and immunology facilitated the understanding of the pathogenesis of lymphoma. Although there are some basic prognostic indicators, the prognostic importance of genetic factors is gradually increasing.

Methods: Fifty six patients who underwent genetic analysis before therapy, were evaluated retrospectively. Deparaffinized lymph node sections were used. Patients were divided into two groups according to the presence of genetic disorder. The groups were compared in terms of clinical features, laboratory parameters and survival.

Results: Genetic abnormality was present in 33 of 56 patients. The most common genetic abnormality was MYC/IGH2 rearrangement. There was no difference between the survival of the groups. There was a positive correlation between B symptoms and the presence of genetic disorder and a negative correlation between leukocyte count and the presence of genetic abnormality.

Conclusion: Despite including small number of patients and heterogeneous histological NHL subtypes, our study may provide contribution to the frequency and type of genetic abnormalities in patients with NHL in Turkey.

Keywords: Non-Hodgkin lymphoma, genetics, FISH, prognosis

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Numerous complex genetic abnormalities affecting proto-oncogenes and tumor suppressor genes take role in the pathogenesis of lymphoma. When the genome of the lymphoma types is examined, it is detected that there are several or sometimes single chromosomal disorders seen as non-random balanced chromosomal translocations.[1] In addition to these translocations; by means of techniques such as fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH), unbalanced chromosomal changes in a number far more than previously known can be detected in lymphoma. These unbalanced chromosomal abnormalities detected at the time of diagnosis increase with disease progression.[2]

Genetic abnormalities detected at the molecular level in lymphomas are inactivation of the tumor suppressor genes resulting from chromosomal translocations and chromosomal deletion or mutation. In some lymphoma subtypes, changes in the genome of lymphoma occur due to onco-

Address for correspondence: Tuba Bulduk, MD. Gulhane Egitim ve Arastirma Hastanesi Hematoloji Bolumu, Istanbul, Turkey
Phone: +90 506 251 24 33 **E-mail:** tuba.kiraz@hotmail.com

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genic viruses. Point mutations may also cause a change in epigenetic regulation of transcription or translation.[3-5]

It was found that the chromosomal abnormalities in non-Hodgkin lymphoma (NHL) were associated with histological subtype,[6-9] immunophenotype,[10] tumor grade,[11,12] response to treatment, and patient prognosis.[13-18] When some specific chromosome translocations were compared with histological classification it was shown that predicting prognosis according to chromosomal abnormalities may be more valuable to improve diagnostic reliability and treatment.[19]

In the present study the association between clinical features and FISH results of the patients with NHL were investigated.

Methods

Fifty six patients whose diagnosis of NHL was histologically confirmed and who underwent genetic analysis before therapy between the years 2013 and 2017, were evaluated. Deparaffinized lymph node sections were used for genetic analysis and fluorescent in situ hybridization (FISH) analysis was performed. Patients were divided into two groups according to the presence of genetic disorder. The groups were compared in terms of clinical features and laboratory parameters at the time of diagnosis, and survival. Survival time was calculated as the time between diagnosis and death or the time between diagnosis and data collection. The study was approved by local Ethical Committee and is in accordance with the current version of the Helsinki Declaration.

FISH Analysis

Interphase FISH analysis was performed using Break Apart Rearrangement DNA Probes, CEP DNA Probes, Dual Color Dual Fusion Translocation DNA Probes, Single or Dual Color Locus Specific DNA Probes and were used to detect the abnormalities according to the manufacturer's (ZytoVision) protocols. The slides were analyzed using an Olympus BX61 fluorescence microscope (Olympus, Tokyo, Japan) and images were captured with a charge-coupled device camera using an image analysis system (Applied Imaging, Grand Rapids, MI). At least 50 e 100 nuclei were examined for each probe whenever possible. The signals derived from the target gene or chromosome centromere were calculated. The cut-off values for each probe were as follows: 10% Break Apart Rearrangement DNA Probes, CEP DNA Probes set and 10-12% Dual Color Dual Fusion Translocation DNA Probes.

Statistical Analysis

All statistical analyses were performed by using SPSS v.25.0 (IBM Corp., Armonk, NY, USA). Data are expressed either as frequencies (n), mean±standard deviation (x±sd) and median 25%-75% quartiles (Median (Q1-Q3)). Shapiro-Wilk's test was used and a histogram and Q-Q plot were examined to assess the data normality assumption. Levene's test was used to assess the variance homogeneity. An independent sample t test was applied to compare the differences between groups for continuous variables hemoglobin (g/dl) and platelet count (/uL). Mann Whitney U test was used to compare other continuous measurements of patients with and without genetic disorders. Log-rank test was used to compare survival times (months) of patients with and without genetic disorders. Pearson Exact Chi-square test was performed on categorical data comparison of the patients with and without genetic disorders according to NHL subtypes. Spearman correlation analysis was applied to determine the relationship between leukocyte count and the presence of genetic disorder, B symptoms and the presence of genetic disorder respectively. A p-value <0.05 was considered statistically significant.

Results

The patients' mean age at the time of diagnosis was 56 (19-85) years. Thirty patients were male and 26 were female. Histopathological diagnoses were diffuse large B-cell lymphoma (DLBCL) (n=25), marginal zone lymphoma (MZL) (n=10), small lymphocytic lymphoma (SLL) (n=5), Burkitt lymphoma (BL) (n=5), follicular lymphoma (FL) (n=5), mantle cell lymphoma (MCL) (n=4) and peripheral T-cell lymphoma (PTCL) (n=2). Since 8 patients did not receive treatment, 7 patients died before evaluation and 3 patients were still being treated at the time of data collection it was not possible to assess remission status in 18 patients. When the study was completed 41 of 56 patients were alive.

Genetic abnormality was present in 33 of 56 patients. The most common genetic abnormality was MYC/IGH2 rearrangement. Eight patients were followed-up without treatment and others were treated with standard treatments according to the diagnosis. The characteristics of patients with and without genetic disorders are given in Table 1.

There was no difference between the survival of the groups with and without genetic abnormalities (p=0.965). There was also no difference between the groups with and without genetic abnormalities in terms the subtype of lymphoma (p = 0.217) (Table 2). The genetic abnormalities detected in our patients were summarized in Table 3.

It was found that there was a positive correlation between B symptoms and the presence of genetic abnormality (r=0.3304, p=0.023), and a negative correlation between leukocyte count and the presence of genetic abnormality (r=-0.303, p=0.023).

Table 1. Comparison of the characteristics of patients with and without genetic abnormalities

Patients' Characteristics	Patients with Genetic Abnormality (n=33)	Patients without Genetic Abnormality (n=23)	p
Age (year)	52.97±18.47	62.17±12.58	0.067
Hemoglobin (g/dl)	12.4 (11.1-13.4)	12.4 (10.3-14.4)	0.815
White blood cell count (x10^9/L)	8.64±9.48	9.36±4.4	0.025
Absolute neutrophil count (x10^9/L)	5.61±7.7	6.42±3.34	0.018
Absolute lymphocyte count (x10^9/L)	2.15±3.81	2.06±2.87	0.868
Platelet (x10^9/L)	232 (182.5-312.5)	247 (152-304)	0.825
Sedimentation rate (mm/hour)	38.76±27.74	39.35±28.33	0.887
C reactive protein (mg/dL)	3.09±5.65	3.24±3.96	0.617
β2 microglobulin (mg/dL)	0.35±0.23	0.53±0.91	0.783

Table 1. Comparison of the characteristics of patients with and without genetic disorders (Continued)

	Patients with genetic disorders (n=33)	Patients without genetic disorders (n=23)	p
Aspartate aminotransferase (U/L)	31.21±31.27	27.22±30.53	0.193
Alanine aminotransferase (U/L)	20.39±20.17	22.91±18.68	0.848
Alkaline phosphatase (U/L)	149.88±185.71	91.3±42.06	0.208
Gamma-glutamyl transferase (U/L)	70.82±147.17	36.87±36.34	0.516
Lactate dehydrogenase (U/L)	614.12±1003.69	394.22±196.07	0.881
Total bilirubin (mg/dL)	0.7±0.86	0.54±0.27	0.635
Albumin (g/dL)	3.96±0.66	4±0.74	0.933
Calcium (mg/dL)	9.31±0.7	9.2±0.6	0.652
Phosphorus (mg/dL)	3.53±0.72	3.64±0.97	0.900
Uric acid (mg/dL)	5.62±2.38	6.39±2.33	0.162
Creatinine (mg/dL)	0.98±0.64	0.88±0.24	0.543
Survival time (month)	31.73±4.87	37.09±3.46	0.965

Table 2. Comparison of the patients with and without genetic abnormalities according to NHL subtypes

Diagnosis	Patients with Genetic Abnormality (n=33)		Patients without Genetic Abnormality (n=23)		Total (n=56)		
	n	%	n	%	n	%	Statistics; p
DLBCL	15	45.5	10	43.5	25	44.6	χ^2=8.485; p=0.210
FL	2	6.1	3	13.0	5	8.9	
SLL	2	6.1	3	13.0	5	8.9	
MCL	2	6.1	2	8.7	4	7.1	
BL	5	15.2	0	0.0	5	8.9	
MZL	7	21.2	3	13.0	10	17.9	
PTCL	0	0.0	2	8.7	2	3.6	

DLBCL: Diffuse large B-cell lymphoma lenfoma. FL: Follicular lymphoma; SLL: Small lymphocytic lymphoma; MCL: Mantle cell lymphoma. BL: Burkitt lymphoma; MZL: Marginal zone lymphoma; PTCL: Peripheral T-cell lymphoma.

Discussion

The advances in molecular biology, cytogenetics and immunology facilitated the understanding of the pathogenesis of lymphoma. Although the age, stage, performance status and some laboratory parameters are used as basic prognostic indicators, the prognostic importance of genetic factors is gradually increasing.

Studies investigating the association between NHL cyto-

Table 3. Distribution of genetic mutations detected in the patients according to lymphoma subtypes

Genetic disorder	DLBCL	FL	SLL	MCL	BL	MZL	Total
BCL6; der(3)(q27.3)	8		1				9
t(8;14)(q24;q32)	6				5		11
t(14;18)(q32;q21.33)	7	2					9
del(7)(q)/-7	1					4	5
+12	1		1				2
+3							0
+8	1						1
t(14;18)(q32;q21.31-21.32)							0
t(11;14)(q13.3;q32)	1			2		1	4
del(5)(q)/-5							0
IGH; der(14)(q32)	1					1	2
TP53; del(17)(p13.1)							0
ATM; del(11)(q22.3)							0
del(13)(q14.2)/del(13)(q34)						2	2
MYB; der(6)(q22.2-22.3)						1	1
+18						1	1

DLBCL: Diffuse large B-cell lymphoma lenfoma. FL: Follicular lymphoma. SLL: Small lymphocytic lymphoma. MCL: Mantle cell lymphoma; BL: Burkitt lymphoma. MZL: Marginal zone lymphoma; *No genetic mutation was detected in peripheral T-cell lymphoma subtype.

genetics and clinical characteristics are limited due to the cytogenetic subgroups consisting small number of patients,[14,20] different histology and tumor grade[13,14,18] or results obtained after cytotoxic therapy.[13,16,20] In our study all patients were analyzed before cytotoxic therapy and there was no effect of the treatment. However, repetition of genetic investigations before and after treatment and/or in case of relapse may provide additional information.

The association between the histological and immunophenotypic subtypes of NHL and the chromosomal abnormalities was reported in large series.[[1,7,8,10,11,16,20] In many studies, it was shown that there were differences between the cytogenetic subgroups of NHL in terms of response to treatment, survival and clinical behavior.[13,14,17,18] Some complex changes in chromosome karyotype and some specific genetic abnormalities such as t (8; 14) (q24: q32) and +7 are negative prognostic factors for NHL.[21,22] It was also shown that t (14; 18), chromosome 7 abnormalities and polyploid chromosomes have the most important effect on the survival of NHL patients.[23]

In our study; the most common genetic abnormalities were MYC/IGH2 (n=11), BCL6 (n=9), BCL2/IGH (n=9) and CEP7/7q31 (n=5). No association was found between of the presence of genetic abnormalities and both the clinical characteristics and survival.

Similarly; in a study performed on 898 patients with DLBCL, it was found that the presence of BCL6 alone or the coexistence of BCL6 with MYC translocation did not have effect on survival and was not related with poor prognosis.[24]

In a study performed on 205 patients with DLBCL it was concluded that MYC positivity alone did not affect the prognosis, but it resulted a more negative course in double hit or triple hit lymphoma.[25] Gong et al.,[26] observed poor prognosis and survival in DLBCL patients who had positivity of MYC, BCL6 and BCL2. This triple positivity was not detected in our patients.

In another study performed on 61 DLBCL patients without double-/triple-hit lymphoma, MYC translocation was associated with overall survival in log-rank test but BCL2 and BCL6 translocations were not prognostic indicators. In the multivariate analysis; the expression of MYC and BCL2 was found to be an independent prognostic factor but BCL6 was not associated with survival.[27]

Offit et al.[1] found no correlation between cytogenetic results and survival in 149 low-grade lymphoma patients. However; in 205 patients with DLBCL abnormal karyotype at diagnosis, especially broken chromosomes in 1q21-23 or the marker chromosomes more than 4, were found to be associated with shortened survival.

Ikegami et al.,[28] found that relapse and progression decreased and the progression-free survival (PFS) increased in patients with IGH translocation, but IGH translocation was not the indicator of a better mean survival in patients with DLBCL in small bowell.

In a study investigating 39 gene mutations in 24 patients with follicular lymphoma, it was detected that HVCN1 mutation was associated with better PFS and CREBBP muta-

tion was associated with lower PFS.[29] Cardenas et al.,[30] determined that the activated B-cell subtype of DLBCL was dependent on BCL6 and this oncogene should be specifically targeted.

The most important limitations of our study were the small number of patients, evaluation of the selected patients whose paraffin blocks were analyzed by genetic department, lack of conventional cytogenetic and molecular results, the heterogeneity in terms of histology and the relatively short follow-up period. Some abnormalities are known to contribute to diagnosis and as expected all of our patients with Burkitt lymphoma had genetic abnormality. The reason for not finding an association between the clinical caharacteristics and genetic disorders can be the differences in staging, treatment and response evaluation due to histological heterogeneity of our patients.

The combination of clinical, immunophenotypic and cytogenetic prognostic markers can better describe group of patients who will benefit better from supportive therapy or more intensive treatment. In acute and chronic leukemias, the detection of same structural chromosomal abnormality as in the Philadelphia chromosome does not have the same biological significance. Thus, the same structural chromosomal changes in different subtypes of NHL may also cause different effects. The effects of genetic findings on prognosis and survival in lymphoma may be evaluated more clearly by large-scale prospective studies in which specific subgroups of NHL were included and in which uniform treatment approaches were used.

Since the epidemiology and geographical distribution of NHL varies between countries, the development of individualized treatment strategies based on prognostic parameters is critical to increase treatment efficacy and patient survival. Therefore, the genetic analysis of patients may provide a new approach to individualized treatment or to the optimization of treatment and also it may lead to the development of new treatment strategies. In our study, only two parameters were associated with the presence of genetic abnormalities. These associations were a positive correlation between the presence of B symptoms and the presence of genetic disorder (r=0.304, p=0.023) and a negative correlation between the number of leukocytes and the presence of genetic disorder (r=-0.303, p=0.023). These results may support the poor prognostic effect of genetic abnormalities however, our data is insufficient to make a definitive conclusion.

Conclusion

In conclusion, despite all limitations, our study may provide an important contribution to the frequency and type of genetic abnormalities in patients with NHL. Although new methods such as next generation sequencing seems more promising, FISH is still the most commonly used methodology for genetic assesment in many centers and provides important prognostic data. In terms of the effect of genetic disorders on prognosis, there is a need for large-scale studies that have longer follow-up periods and include more homogeneous histological subtypes.

Disclosures

Ethics Committee Approval: The study was approved by local Ethical Committee and is in accordance with the current version of the Helsinki Declaration.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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