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Frequency of Paroxysmal Nocturnal Hemoglobinuria in Patients with Lymphoma

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Abstract

Objective: To investigate the frequency of paroxysmal nocturnal hemoglobinuria (PNH), an acquired clonal hematopoietic stem cell disease resulting in complement-mediated hemolysis, in patients with lymphoma by flow cytometry.

Methods: Fifty patients with lymphoma who were admitted to the hematology clinic, newly diagnosed and not yet treated were included in this study conducted in 2014. The presence of PNH clones was checked by FLAER flow cytometry method in peripheral blood samples. FLAER is a non-hemolytic fluorescently labeled inactive toxin aerolysin method that can detect up to 0.5% of PNH cells instead of bacterial toxin aerolysin, which binds to RBCs via the GPI anchor and initiates hemolysis for PNH screening or PNH clone detection. With this technique, PNH clones in all hematopoietic cell lines can be detected in an assay.

Results: PNH clone was observed over 10% in two patients, one male and the other female. However, no hemolysis was found in patients with PNH clones. The lymphoma subtypes of the patients with positive PNH clone were B-cell small-cell lymphocytic lymphoma in the male patient and primary splenic lymphoma in the female patient.

Conclusion: PNH or PNH-like disorders accompanying hematological malignancies, especially lymphomas, are not very common in the literature. There is a need to elucidate the relationship between hematological malignancies and PNH with the help of more advanced molecular techniques.

Key words: Paroxysmal nocturnal hemoglobinuria, PNH, lymphoma, cytometry, FLAER

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INTRODUCTION

Lymphoma is a group of malignant lymphoproliferative diseases arising from different lymphoid tissue cells, primarily B and T cells in the lymphatic system (1). Lymphoma is the most common form of hematological malignancies in developed countries. The classification, which was previously accepted as Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) and changed several times, was lastly revised by the World Health Organization (WHO) in 2016 (2). More than 90 lymphoid neoplasms with different histopathology and heterogeneous behavior are divided into five main categories: "mature B-cell", "mature T and NK cell", "Hodgkin lymphoma", "posttransplant lymphoproliferative disorders" and "histiocytic and dendritic cell neoplasms".

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal hematopoietic stem cell (HSC) disease that can affect all three blood cell lines. The defect in PNH is a somatic mutation of the Xp22.1 gene, called pig-A, located on the short arm of the X chromosome. As a result of this mutation, the binding protein to the cell membrane is impaired and a chronic, uncontrolled complement activation and intravascular hemolysis process begins due to the defect in the cell membrane. Morbidity and mortality depend on cytopenia, thrombophilia and secondary transformations. A better standard of living and prognosis have been achieved with targeted agent therapy (eculizumab) that stops hemolysis (3).

It has been reported that PNH may accompany other hematological diseases (4). Many patient groups with PNH have a history of diagnosis of aplastic anemia (AA) or myelodysplastic syndrome (MDS). In addition, it can progress or transform into some diseases such as AA, leukemia as well as with other diseases (5).

In this study, PNH clone was examined in patients who applied to the Hematology clinic and were found to have lymphoma in their workups. It is aimed to determine the frequency of PNH, which may have similar findings and complications with lymphoma.

METHODS

Patient Selection

Patients who applied to department of Internal Medicine, Hematology clinic between March 2014 and June 2014 and were newly diagnosed with lymphoma were included in the study.

Exclusion criteria were pediatric age group, geriatric patients over the age of 80, those with severely impaired general condition, those who were excluded from the diagnosis of lymphoma during their follow-up, and those who did not want to be included in the study.

Patients were staged according to the Costwold staging classification. For clinical staging, patients' histories were taken, and physical examinations were performed. Blood count and biochemical results were taken into account in patient follow-ups. Again, radiological examinations for the diagnosis of lymphoma were examined.

Collection and Transport of Blood Samples

Since glycosylphosphatidylinositol (GPI) anchor differentiation in the bone marrow is at different stages, peripheral blood sampling was preferred to diagnose PNH by Fluorescein-labeled proaerolysin (FLAER) (6). The blood samples taken were sent to the laboratory of the department of Immunology, and

the samples were studied with the FLAER on the same day.

Experiment Method

PNH clones were studied by 4-color flow cytometry (FCM) with FLAER and analyzed with the FACSDiva Version 6.1.2 data program.

To detect GPI-AP expression:

• For neutrophils, FLAER (Alexa)/CD24 (PE)/CD15 (PerCP)/CD45 (PeCy7) antibody,

• For monocytes, FLAER (Alexa)/CD14 (PE)/CD64 (PerCP)/CD45 (PeCy7) antibody,

• For erythrocytes, CD235a (FITC)/CD59 (PE) antibody was used.

PNH clone values of 10% and above were considered positive for the diagnosis of PNH.

Statistical analysis

Numerical data are shown as mean and standard deviation.

RESULTS

Fifty newly diagnosed lymphoma patients who applied to department of Internal Medicine, Hematology clinic between March 2014 and June 2014 were included in the study.

The youngest case in the study was 24 years old, the oldest case was 76 years old, and the mean age of these cases was 55.66 (Table 1). Of the cases, 22 (44%) were female and 28 (56%) were male (Table 2).

The lymphoma subtypes and numbers of the cases were determined by their clinical records (Table 3), (Figure 1). Accordingly, 33 (64.70%) B-cell lymphomas, 6 (11.76%) T-cell lymphomas, 12 (23.52%) Hodgkin lymphomas, and 0 (0%) others were determined.

Table 1. Age information of the cases

n	Lowest Age	Highest Age	Average Age	Standard Deviation
50	24	76	55,66	15,74

Table 2. Gender information of the cases

Sex	n	Ratio (%)
Female	22	%44
Male	28	%56
Total	50	%100

Table 3. Lymphoma subtypes and their numbers

Lymphoma Type	N	Ratio (%)
B cell lymphoma	32 (13)	64
T cell lymphoma	6 (3)	12
Hodgkin lymphoma	12 (6)	24
Histiocytic and dendritic lymphoma	0	0
Posttransplant lymphoproliferative disorders	0	0

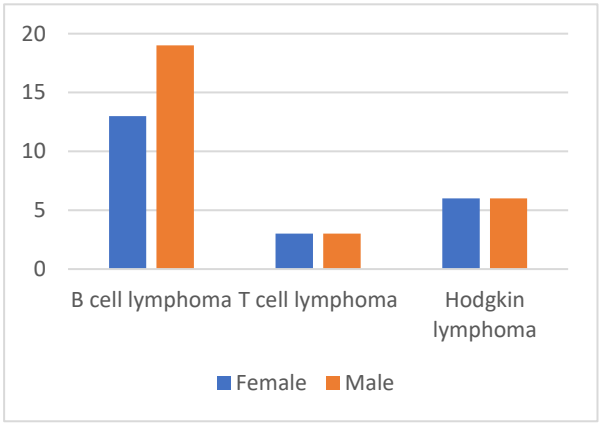

Figure 1. Distribution plot of lymphoma subtypes by gender

In FLAER flow cytometric analysis, PNH clone was observed as > 10% in 2 out of 50 cases, one male and one female (Table 4). The rate of patients with positive PNH clone was 4%. The FLAER results of two patients are shown in Figure 2 and 3. Hemolysis was not found in patients with positive PNH clone. The lymphoma subtypes of the patients with positive PNH clone were B-cell small-cell lymphocytic lymphoma in the male patient and primary splenic lymphoma in the female patient.

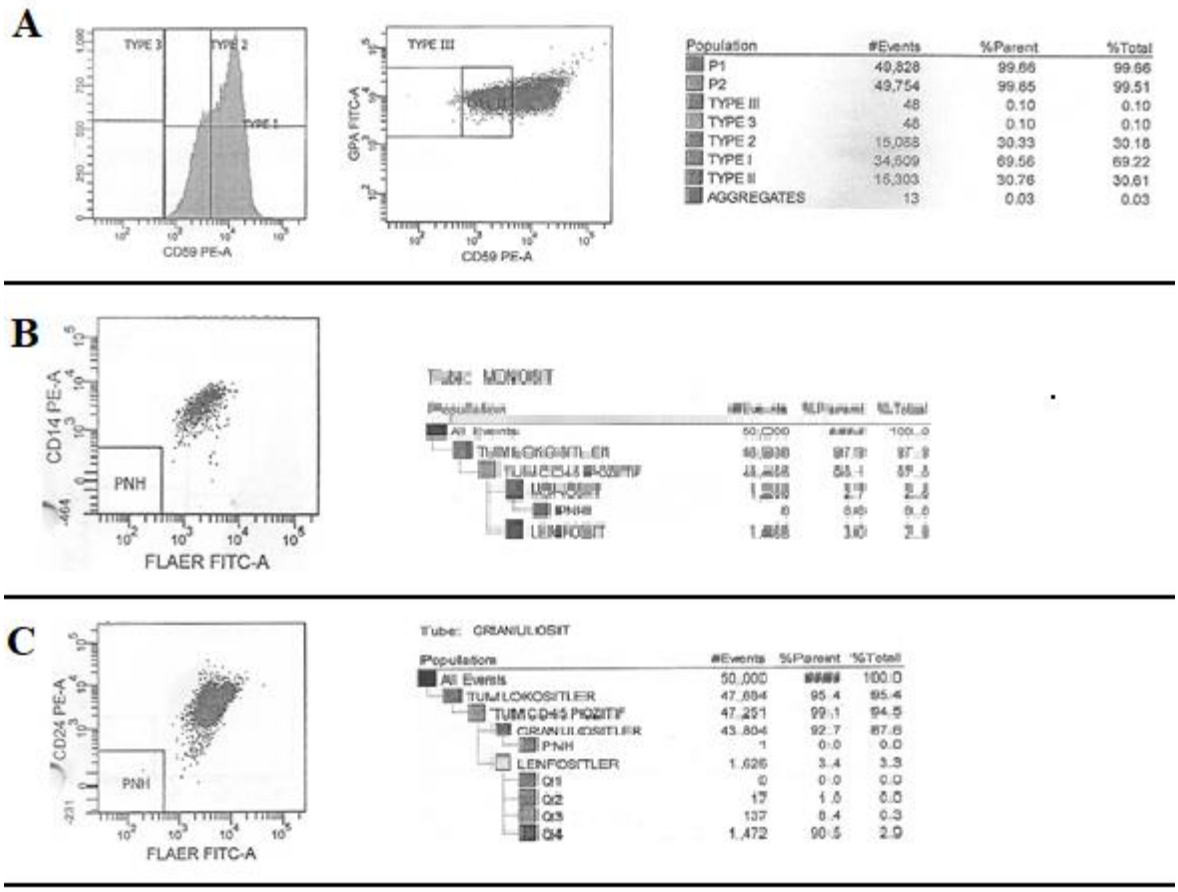

Figure 2. FLAER erythrocyte (A), monocyte (B) and granulocyte (C) results of the first patient (male) with PNH clone

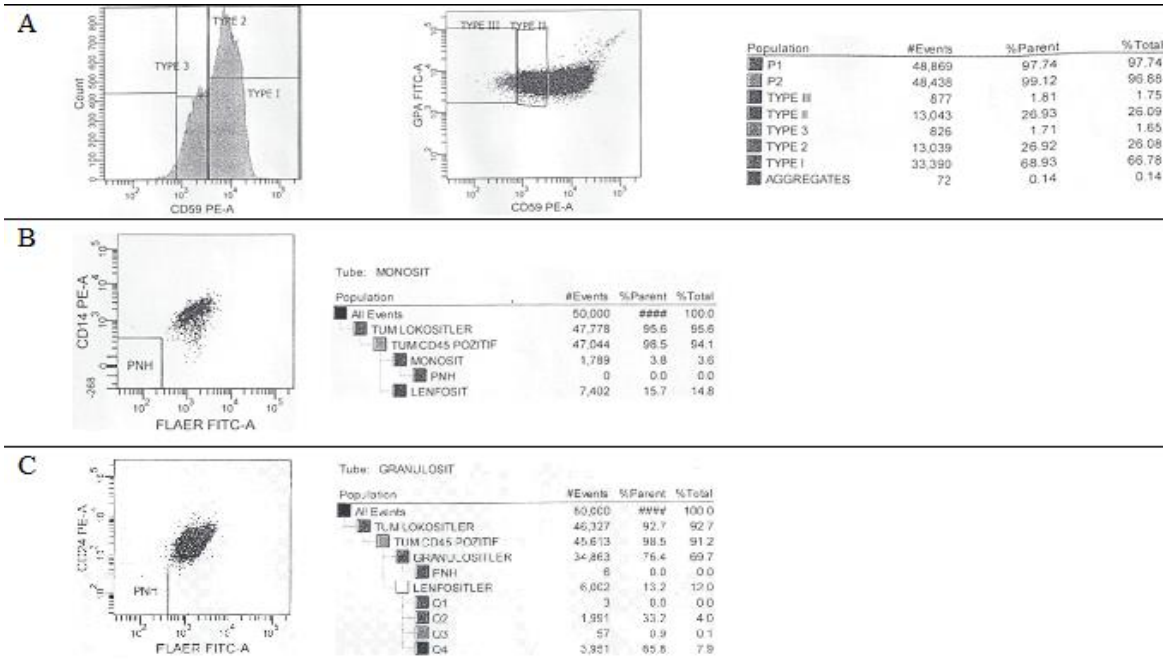

Figure 3. FLAER erythrocyte (A), monocyte (B) and granulocyte (C) results of the second patient (female) with PNH clone.

Anemia and thrombocytopenia were evident in the complete blood count (CBC) of the first patient with PNH, a male patient, while the CBC of the second female patient had only mild anemia and thrombocytosis (Table 5).

Table 4. Clone levels in erythrocytes of patients with positive PNH clone

PNH clone	First patient	Second patient
Type II	% 30,33	% 26,08
Type III	% 0,1	% 1,75
Total	% 30,61	% 28,8

Table 5. Complete blood count and some biochemical values of patients with PNH.

CBC and Biochemistry Values	1th Patient	2nd Patient
White blood cell (WBC) ($x10^3$/uL)	6,3	21,4
Hemoglobin (HGB) (g/dL)	8,97	11,3
Hematocrit (HCT) (%)	26,1	34,6
Thrombocyte (PLT) ($x10^3$/uL)	57,9	709
Creatinine (mg/dL)	0,78	0,67
Total Bilirubin (mg/dL)	1,8	0,2
Direct Bilirubin (mg/dL)	1,2	0,1
Lactate dehydrogenase (LDH) (U/L)	267	394
Uric acid (mg/dL)	3,7	5,8
C-reactive protein (CRP)	7,96	6,99

DISCUSSION

PNH clone can be seen with many hematological clonal diseases. In studies, the association of bone marrow, which is one of the organs of the hematopoietic system, and disease groups arising from it, and PNH or PNH-like disorder took place. On the other hand, there are a few studies coexistence of PNH and lymphoma, a disease of the lymphatic system, which is another organ of the hematopoietic system.

Unlike leukemic transformation in patients with PNH, PNH-like deficiencies of CD55 and CD59 have been reported in acute leukemias (7,8). Although rare, PNH-like cell defects have been detected in chronic myeloproliferative diseases, chronic myeloid leukemia (CML) and Philadelphia chromosome negative chronic myeloproliferative diseases (9–11).

Detection of PNH clones in myelodysplastic syndrome is better known and is more common than other hematological diseases (12–14). However, myelodysplastic findings in the bone marrow can also be observed in PNH patients. Therefore, the relationship between both diseases is mixed and controversial (14). The presence of PNH together with lymphoproliferative diseases is an extremely rare condition. There are case reports or presentations in which a few cases are collected in the literature (15–17). In different lymphoproliferative disease types, different rates of PNH clones can be detected in untreated patients, as well as in patients who have received some special treatments such as anti-CD52 antibody (CAMPATH-1H) (18,19). Fukuda et al. showed complete loss of CD55 (type III clone) in two of 10 patients with non-Hodgkin lymphoma, but not in any of the 6 patients with chronic lymphocytic leukemia (CLL) (20). Seya et al. reported that CD55 loss may occur in NHL patients rather than other hematological malignancies (21).

In the largest systematic study available in the literature, investigating the PNH clone in 195 patients with lymphoproliferative disease, most of whom had CLL, the double negative rate of CD55 and CD59 in erythrocytes was found to be 9.2%. Isolated CD55 and CD59 negativity rates were found to be 8.7% and 0.9%, respectively. CD59 expression appears to be

better preserved in these patients. Researchers stated that none of these patients had signs of hemolysis (13). The reason for the absence of hemolysis, unlike that seen in classical PNH, can be explained by the decreased expression of GPI-anchor proteins in a small population of red blood cells, which is not sufficient for hemolysis. It has also been suggested that CD55 deficiency alone is not sufficient for hemolysis (22).

Similarly, there were no signs of hemolysis in patients with positive PNH clones in our study. In the first patient (KD), whose PNH clone was detected by evaluating CD59 expression in erythrocytes, partial loss of CD59 (type II clone) was detected at a rate of 30.33%. A severe loss of CD59 (type III clone) was detected at a rate of 0.1%. In the second patient (DS), the type II clone rate was 27% and the type III clone rate was 1.8%. CD55 level was not evaluated in our study.

We found PNH-like disorder in 4% of all lymphoma patients. This is slightly lower than that found in previous studies of lymphoproliferative diseases (13,20).

The fact that only CD59 was investigated in our cases and the CD55 level was not measured suggests that some cases that may indicate low CD55 may have been overlooked.

Again, in the study of Meletis et al., CD55 and CD59 deficiency was observed at a higher rate in low-grade B-cell NHLs than in other NHL types (13). In our study, lymphomas of both patients were low grade but advanced.

In this prospective study, we used a relatively new flow-cytometric method, FLAER, to detect the PNH clone. In previous similar studies, CD55 and CD59 expression levels were used by conventional flow-cytometry, and Ham and acid sucrose lysis tests were used in previous studies and case reports.

Consistent with most case reports and studies in the literature, PNH clone was not detected in neutrophils and monocytes. The PNH-like disorder in our study concerned only erythrocytes.

PNH clone was detected in lymphocytes in two separate studies; GPI deficiency was detected in T cells in CLL patients who were administered anti-CD52 antibody (CAMPATH-1H). This abnormality is explained as anti-CD52 antibody binding to CD52, a GPI-dependent protein, may lead these cells to apoptosis, causing T lymphocytes with low GPI levels to gain clonal advantage and increase them. (18,19). Indeed, it has been shown that CD55 and CD59 expression are decreased in B and T lymphocytes after administration of CAMPATH-1H in in-vitro cultures (23).

Interestingly, PNH or PNH-like erythrocytes with CD55 and CD59 deficiency are more commonly detected in patients with CLL and low-grade NHL. In one study, the rate of erythrocytes with decreased CD55 and CD59 expression was found to be less than 10% in different lymphoproliferative disease groups. In PNH disease, the decrease in the expression of these proteins is usually greater. Therefore, it is understood that the PNH clone, which can be detected rarely in hematological diseases, does not indicate a true PNH disease. We can say that this is a PNH-like side finding that is not reflected in the clinic or a laboratory finding that may accompany it. The absence of clinical findings of PNH in patients with PNH clones in the study by Meletis et al. supports this thesis. (13). In the same study, CD55 and CD59

deficiency were also found in erythrocytes in patients with nodular sclerosing subtype in classical Hodgkin lymphoma.

Other hypotheses have also been proposed to explain the presence of the PNH phenotype in lymphoproliferative diseases and other clonal diseases. Somatic mutations developing in stem cells in lymphoproliferative diseases may also cause other somatic mutations. It is thought that the PNH clone is usually found in such low amounts that it cannot be detected in normal individuals. During the course of the lymphoproliferative disease, the PNH clone can reach a detectable level, perhaps by gaining a survival advantage through an immune mechanism.

In a study by Fukuda et al., CD55 expression level was found to be low in only 2 NHL patients out of 38 people with most lymphoproliferative diseases, including 6 patients with chronic myeloid leukemia. In the related study, the presence of CD55 expression was examined in mononuclear cells instead of erythrocytes. For this reason, it has been explained by researchers that there may be loss of CD55 in lymphoma cells (20). This finding is not consistent with the two cases in our study in which CD59 deficiency was detected only in erythrocytes. As reported in a case report, when unexpected cytopenia, thrombosis or hemolysis develops in a patient with lymphoma, it should be kept in mind that PNH disease or a PNH-like disorder may coexist. (16).

The disappearance of the PNH-like clone in erythrocytes after chemotherapy in one of the two cases in our study is also interesting in that it shows that this disorder occurs in association with lymphoma. A similar finding was not mentioned in other studies and case reports.

It is important to differentiate PNH disease from the PNH-like disorder found in other clonal diseases. Hemolysis did not occur in the increase of PNH clones in PNH-like disorder or with another hematological disease, as observed in other studies and the cases in our study. This can be interpreted as the absence of classical PNH findings, since the PNH clone is found at a very low level in these diseases.

The fact that the methods used in the studies and case reports are different, and the lack of studies with FLAER makes it difficult to compare our results with others. Further and standardized studies with the help of flow-cytometry and molecular techniques are necessary to elucidate the relationship between hematological malignancies and PNH and to develop new treatment methods.

CONCLUSION

PNH is a disease that characterized by episodes of hemolysis, thrombosis, and cytopenias associated with bone marrow failure.

In our study, we detected positive PNH clone in two (4%) of 50 patients. According to the literature, the presence of PNH clones in patients with lymphoma in our study is less frequent. No signs of hemolysis were observed in patients with positive PNH clones.

Although PNH or PNH-like disorder is not common in patients with lymphoma, it is a pathology that should be considered. It should be known that it can be seen without hemolysis and may accompany hematopoietic diseases. It should also be kept in mind that the PNH clone may appear later and may disappear spontaneously or after treatments (such as chemotherapy).

Performing PNH clone presence and frequency studies with a new technique, FLAER, and in larger patient groups will provide more accurate results and will allow better elucidation of the molecular and clinical association of lymphoma and PNH.

Ethics Committee Approval: Ethics committee approval was received for this study from Kocaeli University Clinical Research Ethics Committee. (Date: 03.18.2014; Decision no: 6/2)

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: A H Design: AH, M A; Data Collection and Processing: M O, Analysis or Interpretatio, Writing: A T V. M O Critical review: A H

Conflict of Interest: No conflict of interest was declared by the authors.

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