



The Prevalence of Paroxysmal Nocturnal Hemoglobinuria Clone in Adult Patients with Idiopathic Pulmonary Hypertension

İdiyopatik Pulmoner Hipertansiyonu Olan Erişkin Hastalarda Paroksizmal Noktürnal Hemoglobinüri Klonu Prevelansı

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Abstract

Aim: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder that may lead to several conditions such as thromboses, Budd-Chiari syndrome, renal failure, impotence, and pulmonary hypertension (pHT). Since the presentation of PNH may be occult, monitoring for clonal evolution is recommended in certain situations including aplastic anemia, Myelodysplastic syndrome, and unexplained cytopenia, and thrombosis. The prevalence of PNH clone in patients with idiopathic pHT is unknown. We designed a study to determine the prevalence of PNH clone in patients with idiopathic pHT, since it may be the first isolated presentation of the disease.

Methods: A total of 45 patients with pHT were screened for PNH clone by proaerolysin conjugated with fluorescein.

Results: Only two out of 45 patients had elevated lactate dehydrogenase (LDH) levels at presentation. PNH clone was detected in none of the patients.

Conclusion: Screening for PNH clone in patients with pHT, who have normal LDH levels is unnecessary.

Keywords: Paroxysmal nocturnal hemoglobinuria, pulmonary hypertension, clone

Öz

Amaç: Paroksizmal noktürnal hemoglobinüri (PNH) tromboz, Budd-Chiari sendromu, böbrek yetmezliği, impotans, pulmoner hipertansiyon (pHT) gibi çeşitli hastalıklara sebebiyet verebilen klonal bir hastalıktır. PNH'nin prezentasyonu sessiz olabileceğinden, aplastik anemi, Miyelodisplastik sendrom, açıklanamaya sitopeniler ve tromboz gibi çeşitli durumlarda PNH klonu taranması önerilmektedir. İdiyopatik pHT'si olan hastalarda PNH klonu prevalansı ise bilinmemektedir. PNH'li hastalarda pHT hastalığının ilk bulgusu olabileceğinden, pulmoner hipertansiyonu olan hastalarda PNH klonu prevelansını belirlemek amacıyla bir çalışma tasarladık.

Yöntemler: pHT'si olan toplam 45 hastada florasanla konjuge proaerolizin yöntemiyle PNH klonu tarandı.

Bulgular: Kırk beş hastanın yalnızca ikisinde başlangıç laktat dehidrogenaz (LDH) düzeyi normalin üzerindeydi. PNH klonu hiçbir hastada saptanmadı.

Sonuç: LDH düzeyi normal olan pHT hastalarında PNH klonu taranması gerekli görülmemiştir.

Anahtar Sözcükler: Paroksizmal noktürnal hemoglobinüri, pulmoner hipertansiyon, klon

Introduction

Pulmonary hypertension (pHT) is defined as a mean pulmonary artery pressure (mPAP) ≥ 25 mmHg at rest, measured by right heart catheterization. The initial

symptoms of the disease result from an inability to adequately increase cardiac output during exercise. These include exertional dyspnea, lethargy, and fatigue. The progress of pHT leads to development of right ventricular

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hypertrophy and failure, producing right heart failure symptoms. pHT is now more common in patients with hereditary or acquired hemolytic anemia; this is probably related to increased awareness of pHT by physicians. Development of pHT is a poor prognostic indicator in patients with hemolytic anemia (1).

Paroxysmal nocturnal hemoglobinuria (PNH) originates from clonal enlargement of benign hematogenic stem cells that have gained a somatic mutation of the X-chromosome gene Phosphatidylinositol glycan anchor biosynthesis, class A (PIGA). This gene plays a role in the production of the glycosyl phosphatidylinositol (GPI) moiety that synthesizes some proteins to the cell surface. As a result of mutant PIGA, all mature cells (monocytes, erythrocytes, granulocytes, etc.) are deficient in all GPI-anchored proteins (GPI-APs). The clinical signs and the symptoms of PNH are hemolytic anemia, bone marrow (BM) failure and thrombosis (2). Patients may develop pHT and impaired renal function associated with hemoglobinemia in time.

Thrombosis in PNH patients is believed to be multifactorial and not completely understood, but like other symptoms of the disease, it is probably associated with lack of cell surface proteins (GPI proteins) and complement system activation. Indeed, C5a is a proinflammatory mediator and may induce thrombosis. In addition, nitric oxide (NO) reduction (because of intravascular hemolysis and NO consumption) has been related to increased platelet aggregation and adhesion; also related to induced clot formation. Blood cells in PNH have no GPI-anchored urokinase receptor; as a result, fibrinolysis is also affected in PNH. Finally, tissue factor pathway inhibitor, a major inhibitor of tissue factor, has been proven to require a GPI-anchored chaperone protein for trafficking to the endothelial cell surface (3).

Many studies suggest that the reduction in NO content in microcirculation plays a direct role in the pathogenesis of disease-related symptoms through the mechanisms such as vasoconstriction, leukocyte adhesion, platelet activation, endothelial damage, and increased production of free oxygen. NO scavenging can also be the reason for the arterial spasm and related clinical manifestations. Eculizumab is a recombinant humanized IgG2/4 κ monoclonal antibody that binds to the complement C5 protein with high affinity, blocking the degradation of C5 protein to C5a and C5b, thereby preventing the formation of the terminal complement cascade C5b-9. This mechanism shows us the pathophysiology of pHT only in half of the patients. Therefore, other mechanisms besides NO consumption (such as pulmonary embolism) are thought to lead to pHT in PNH patients (4). However, data regarding the prevalence of PNH clone in patients with pHT is scarce.

In this study, we aimed to demonstrate the presence of any underlying PNH clones in patients with pulmonary arterial hypertension and chronic thromboembolic pHT.

Methods

Patients

A total of 45 patients with pHT were recruited in 2015. Age of patients varied between 18-90 years. Possible cause of pHT was idiopathic pulmonary arterial hypertension (IPAH) in 39 patients, chronic thromboembolic pHT in four patients, and pHT associated with connective tissue in two patients. All patients enrolled in the study were diagnosed with pHT by right heart catheterization and followed up by the İstanbul University Cardiology Institute pHT outpatient clinic.

Inclusion and Exclusion Criteria

Inclusion criteria goes as follows: mPAP \geq 25 mmHg at rest measured by right heart catheterization, end-expiratory mean pulmonary capillary wedge pressure $<$ 15 mmHg, and pulmonary vascular resistance $>$ 3 Wood units. Exclusion criteria include mPAP $<$ 25 mmHg at rest measured by right heart catheterization, end-expiratory mean pulmonary capillary wedge pressure \geq 15 mmHg, forced expiratory volume in 1 second/forced vital capacity ratio $<$ 70% in respiratory function tests and/or history of other diseases accompanied by hypoxemia, any known diseases which may result in pHT such as Eisenmenger syndrome, sarcoidosis, myeloproliferative disorder, glycogen storage disease, etc., and any known diagnosis of PNH.

Paroxysmal Nocturnal Hemoglobinuria Clone

PNH clone was detected by proaerolysin conjugated with fluorescein. 2 mL of peripheral blood from patients with pHT was collected into ethylenediaminetetraacetic acid tubes. Presence of PNH clone was studied from peripheral blood in the tubes which were kept at room temperature less than 24 hours. The PNH clone levels were studied within the year before the study.

Patient demographics, complete blood count, lactate dehydrogenase (LDH) levels, PNH clone levels and mPAP were reviewed retrospectively from the patient records.

Study assessments and methods were approved by the Taksim Training and Research Hospital Ethics Committee (dated 23.12.2015/approval no: 31). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients for inclusion and publication of anonymized data.

Statistical Analysis

The Shapiro-Wilk test was used for determining the normal distribution of data. The independent Sample's

t-test (t-test for independent samples) and Mann-Whitney U test were used for group-wise comparisons. Chi-square tests were used for the analysis of categorical data. The data were summarized as mean \pm standard deviation and median (Q1; Q3). A p value of less than 0.05 was considered statistically significant.

Results

Demographics

The median age of 45 studied patients was 51 years (21-83). The minimum age was 21 years and the maximum age was 83 years for the participating patients. Thirty-four patients (75.6%) were female. The patients with IPAH had a mPAP of 57.78 mmHg with a minimum of 26 mmHg and maximum of 115 mmHg. (Table 1).

Paroxysmal Nocturnal Hemoglobinuria Clone

No PNH clone was observed in any patients. As an indicator of hemolysis, LDH levels were evaluated: the IPAH group had a median of 203 U/L (N: 0-248 U/L). Only two patients with IPAH were observed to have a LDH level of two times or above the upper limit of normal.

the most studied group and idiopathic and hereditary pulmonary arterial hypertension is very rare in the general population. Its prevalence is about 5-15 per million adults (5,6).

PNH is a rare, acquired clonal hematopoietic stem cell disorder characterized by chronic intravascular hemolysis findings, BM failure and thrombosis, and the prevalence of the disease was considered to be 1-10 per million (7,8). pHT is also very rare in patients with PNH, yet there are no reports of prevalence. Free hemoglobin produced excessively during intravascular hemolysis may exceed the clearing capacity of haptoglobin, and as a result, a high level of free hemoglobin consumes endogenous NO (9). It also reduces the plasma arginine pool due to hemolysis as well as the increase in production of erythrocyte arginase 1 enzyme (which converts L-tryptophan, a substrate of NO synthesis, to ornithine) and reduces systemic use of NO (10). A decrease in NO level is associated with various sequelae such as development of systemic and pulmonary vascular resistance, pHT and disturbances in endothelial function and smooth muscle tone (9). pHT may occur in patients with PNH secondary to NO depletion in pulmonary circulation and/or due to pulmonary embolism (11). The cause of NO depletion is considered to be associated with intravascular hemolysis ("hemolysis-associated pHT") (12).

Hill et al. (3) performed a study in 2010 on 87 PNH patients to demonstrate the effect of eculizumab treatment on NO depletion associated with hemolysis; dyspnea and pHT. The erythrocyte breakdown was determined by the serum LDH level. The mean LDH levels in the enrolled patients were observed to be 2229 \pm 1025 U/L. A Doppler echocardiography (ECHO) was performed for 28 patients with hemolytic PNH in order to determine their pulmonary artery pressure which was found to be high in 14 of 20 patients with measurable treatment response (70%). Mild to moderate pHT was observed in 12 patients (60%) and moderate to severe pHT was detected in two patients (10%) (13).

In another study by Hill et al. (13) in 2012, 29 patients with hemolytic PNH were examined for their cardiac functions by Doppler ECHO. The median age of the patients was 39.3 years; the median PNH duration was 3.2 years, and the median LDH level was observed to be 7.7 times the normal (mean 3133 \pm 385.6 U/L). The basal granulocyte PNH and erythrocyte PNH clone levels were determined to be 92.1% and 32.5%, respectively. An increased systemic pulmonary artery pressure was detected by Doppler ECHO in 36% of patients. The pHT prevalence of 36% may be explained by the hemolytic nature of the patients and the related significant decrease in NO levels.

The decrease in N-terminal brain-type natriuretic peptide (NT-proBNP) levels in patients for whom hemolysis

Table 1. Clinical and laboratory variables of participants

Variables	IPAH (n=45)
mPAP (mmHg) ^a	57.78 \pm 20.49
6MWT (m) ^a	344.65 \pm 136.23
WBC ($\times 10^3/\mu\text{L}$) ^a	7268.42 \pm 2007.43
HGB (g/dL) ^a	13.16 \pm 2.02
HCT (%) ^a	39.99 \pm 5.54
Age, years ^b	51 (39-61)
Smoking (packs/year) ^b	0 (0-1.5)
MCV (fL) ^b	85.2 (80.22-89.12)
Platelets ($\times 10^3/\mu\text{L}$) ^b	208 (161.25-246.50)
LDH (U/L) ^b	203 (177.25-225.75)
Total bilirubin (mg/dL) ^b	0.62 (0.41-0.79)
Indirect bilirubin (mg/dL) ^b	0.36 (0.23-0.48)
BNP (pg/mL) ^b	770.50 (393.50-2619.50)
PNH clone (%) ^b	0

BNP: Brain natriuretic peptide, HGB: Hemoglobin, HCT: Hematocrit, IPAH: Idiopathic pulmonary arterial hypertension, LDH: Lactate dehydrogenase, MCV: Mean corpuscular volume, mPAP: Mean pulmonary artery pressure, 6MWT: Six-minute walk test, PNH: Paroxysmal nocturnal hemoglobinuria WBC: White blood cell
Data were presented as ^amean \pm standard deviation or ^bmedian (range)

Discussion

pHT is defined as a mPAP \geq 25 mmHg at rest, measured by right heart catheterization (1). The World Health Organization has classified pHT based upon etiology into the five groups. Pulmonary arterial hypertension is

was prevented by eculizumab treatment indicated a relationship between hemolysis and pHT. It was observed that all 11 patients with pHT had increased hemolysis (LDH >2 X normal); no pHT was detected in patients with a LDH of <2 X normal, and the NT-proBNP levels were normal (13). Evidence explaining the frequency of PNH clone in patients with pHT was searched. Our study, to our knowledge, is the first to investigate PNH clone in IPAH and CTEPH patients. No PNH clone was detected in any of 45 enrolled patients.

Hill et al. (13) revealed that pHT was common in PNH patients with elevated LDH levels. In our study, only two patients were on the upper limit of normal LDH level. Our findings therefore suggest that there is no point to screen PNH clone for the etiology of pHT in patients with normal LDH levels.

Conclusion

In the light of current data, routine screening for PNH clone is not recommended for patients with IPAH and chronic thromboembolic pHT who have normal LDH levels and hemolysis tests.

Authorship Contributions

Surgical and Medical Practices: Ü.Y.S., M.S.K. Concept: M.A., Design: T.E. Data Collection or Processing: T.E. Analysis or Interpretation: C.B., T.E., M.A. Literature Search: T.E. Writing: T.E.

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