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

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Abstract

Aim: Paroxysmal nocturnal hemoglobinuria (PNH) a is a clonal disorder that may lead to several conditions such as thromboses, Budd-Chiari syndrome, renal failure, impotence, pulmonary hypertension (pHT). Since the presentation of PNH may be occult, clonal monitoring is recommended in certain situations including aplastic anemia, myelodysplastic syndrome, and unexplained cytopenia’s, and thromboses. The prevalence of PNH clone in patients with idiopathic pHT is unknown.

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

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: In conclusion, screening for PNH clone in patients with pHT, who have normal LDH levels is unnecessary.

Keywords: Paroxysmal nocturnal hemoglobinuria, pulmonary hypertension, clone

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
hypertrophy and failure, producing right heart failure symptoms. pHT is becoming a recognized complication of the hereditary and acquired hemolytic anemias, associated with a poor prognosis (1).

Paroxysmal nocturnal hemoglobinuria (PNH) arises as a result of the nonmalignant clonal expansion of one or several hematopoietic stem cells that have acquired a somatic mutation of the X-chromosome gene PIGA that is required for synthesis of the glycosyl phosphatidylinositol (GPI) moiety that anchors some proteins to the cell surface. As a consequence of mutant PIGA, the progeny of affected mature cells (erythrocytes, granulocytes, monocytes, platelets, and lymphocytes) are deficient in all GPI-anchored proteins (GPI-APs) that are normally expressed on hematopoietic cells and all GPI-APs that are normally expressed on hematopoietic cells are deficient on the progeny of PIGA mutant stem cells. The clinical manifestations of PNH are hemolytic anemia, thromboses, and bone marrow (BM) failure (2). Patients may develop pHT and impaired renal function associated with hemoglobinemia in time.

The mechanism of thrombosis in PNH is not entirely understood and is probably multifactorial, but similar to other manifestations of the disease, it is probably related to the GPI anchor protein deficiency and activation of complement. Indeed, C5a is proinflammatory and may increase the risk for thrombosis. Furthermore, nitric oxide depletion (as a consequence of intravascular hemolysis and nitric oxide scavenging) has been associated with increased platelet aggregation, increased platelet adhesion, and accelerated clot formation. Fibrinolysis can also be perturbed in PNH given that PNH blood cells lack the GPI-anchored urokinase receptor. Lastly, tissue factor pathway inhibitor (TFPI), a major inhibitor of tissue factor, has been shown to require a GPI-anchored chaperone protein for trafficking to the endothelial cell surface (3).

Numerous experimental and clinical studies have focused on the hypothesis that nitric oxide (NO) depletion in the microcirculation plays a central role in the pathogenesis of thrombosis and other manifestations possibly by inducing vasoconstriction, platelet activation, leukocyte adhesion, endothelial damage, oxygen free radical production, and pHT. NO scavenging in PNH patients is also likely to be responsible for clinical manifestations due to arterial spasm. Eculizumab, a recombinant humanized IgG2/4κ monoclonal antibody that specifically binds to the complement protein C5 with high affinity, thereby inhibiting its cleavage to C5a and C5b and blocking the generation of the terminal complement cascade C5b-9, eliminated the ‘evidence’ of pHT in only half the affected cases, suggesting that hemolysis-associated NO scavenging was not the sole cause of the pHT (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 4 patients and pHT associated with connective tissue in 2 patients. All patients enrolled in the study, were diagnosed with pHT by right heart catheterization and followed up by the Istanbul University Cardiology Institute pHT outpatient clinic.

**Inclusion and exclusion criteria**

Inclusion criteria goes as follows: mean pulmonary artery pressure ≥ 25 mmHg at rest measured by right heart catheterization, end-expiratory mean pulmonary capillary wedge pressure < 15 mmHg, pulmonary vascular resistance > 3 Wood units. Exclusion criteria include mean pulmonary artery pressure < 25 mmHg at rest measured by right heart catheterization, end-expiratory mean pulmonary capillary wedge pressure ≥ 15 mmHg, FEV1/FVC rate < 70% in patients’ 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., any known diagnosis of PNH.

**Paroxysmal Nocturnal Hemoglobinuria Clone**

PNH clone was detected by proaerolysin conjugated with fluorescein. 2 ml of peripheral blood of patients with pHT was taken to EDTA tubes. Presences of PNH clone were studied from peripheral blood in the tubes which were kept at room temperature less than 24 hours. The PNH clone was studied within the year before the study.

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

Study assessments and methods were approved by the local Institutional Ethics Committee (dated 23.12.2015/ No:31) and were conducted in accordance with the current version of the Helsinki Declaration. Written informed consent was obtained from all patients for inclusion and publication of anonymized data.
Statistical Analysis

The Shapiro-Wilk test was utilized for determining the normal distribution of data. The independent samples t-test (t-test for independent samples) and Mann-Whitney U test were used for group-wise comparisons. Chi-square tests were utilized for the analysis of categorical data. The data were summarized as mean ± SD and median (Q1; Q3). P<0.05 was considered statistically significant.

Results

Demographics

The median age of 45 studied patients was 51 years (39; 61). 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 mean pulmonary artery pressure 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 of the patients were evaluated: the IPAH group had a median of 203 U/L (N: 0-248 U/L). Only 2 patients with IPAH were observed to have a LDH level of two times or above the upper limit of normal.

Discussion

pHT is defined as a mean pulmonary artery pressure (mPAP) ≥25 mmHg at rest, measured by right heart catheterization (1). The epidemiology for patients with pHT varies by group. Pulmonary arterial hypertension is 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, bone marrow 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 many 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 PNH patient’s secondary to nitric oxide depletion in pulmonary circulation and/or due to pulmonary embolism (11). The cause of nitric oxide depletion is considered to be associated with intravascular hemolysis ("hemolysis-associated pHT") (12).

Hill et al. performed a study in 2010 in 87 PNH patients to demonstrate the effect of eculizumab treatment on nitric oxide depletion associated with hemolysis; dyspnea and pHT levels. The erythrocyte breakdown was determined by the serum LDH level. The mean LDH levels of the enrolled patients were observed to be 2229 ± 1025 U/L. A Doppler echocardiography was performed for 28 patients with hemolytic PNH in order to determine their pulmonary artery pressures and the pulmonary artery pressure 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 2 patients (10%) (13).

In another study by Hill et al. in 2012, 29 patients with hemolytic PNH were examined for their cardiac functions by Doppler echocardiography. The median age of the patients was 39.3 years; the median PNH duration was 3.2 years, and the median LDH levels were observed to

<p>| Table 1. Clinical and laboratory variables of participants |</p>
<table>
<thead>
<tr>
<th>Variables</th>
<th>IPAH (n=45)</th>
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<tbody>
<tr>
<td>mPAP (mmHg)a</td>
<td>57.78±20.49</td>
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<tr>
<td>6MWT (m)a</td>
<td>344.65±136.23</td>
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<tr>
<td>WBC (x10³/µl)a</td>
<td>7268.42±2007.43</td>
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<tr>
<td>HGB (g/dl)a</td>
<td>13.16±2.02</td>
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<tr>
<td>HCT (%)a</td>
<td>39.99±5.54</td>
</tr>
<tr>
<td>Age, yearsb</td>
<td>51 (39-61)</td>
</tr>
<tr>
<td>Smoking (packs/year)b</td>
<td>0 (0-1.5)</td>
</tr>
<tr>
<td>MCV (fL)b</td>
<td>85.2 (80.22-89.12)</td>
</tr>
<tr>
<td>Platelets (x10³/µl)b</td>
<td>208 (161.25-246.50)</td>
</tr>
<tr>
<td>LDH (U/L)b</td>
<td>203 (177.25-225.75)</td>
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<tr>
<td>Total bilirubin (mg/dl)b</td>
<td>0.62 (0.41-0.79)</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dl)b</td>
<td>0.36 (0.23-0.48)</td>
</tr>
<tr>
<td>BNP (pg/ml)b</td>
<td>770.50 (393.50-2619.50)</td>
</tr>
<tr>
<td>PNH Clone (%)b</td>
<td>0</td>
</tr>
</tbody>
</table>

BNP: Brain natriuretic peptide, ES: Eisenmenger’s syndrome, 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 mean ± standard deviation or median (range)
be 7.7 times the normal (mean 3133 ± 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 the 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 NT-proBNP levels of the patients for whom hemolysis 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 < 2 X normal, and the NT-proBNP levels were normal (13). Evidence explaining the frequency of PNH clone in patients with pHT is 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. reveals that pHT is common in PNH patients with elevated LDH levels. In our study, only two patients were on the upper limit of 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 who have normal LDH levels and hemolysis tests with IPAH and chronic thromboembolic pHT.

**Disclosure**

The authors report no conflict of interest.

**References**