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Iron overload: an overlooked complication in classic paroxysmal nocturnal hemoglobinuria.

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Abstract

BACKGROUND: Paroxysmal nocturnal hemoglobinuria (PNH), an acquired clonal bone marrow disorder due to somatic mutation of PIG-A gene, results in deficient expression of both glucosil-phosphatidylinositol (GPI) and proteins anchored to GPI on the surface of blood and hematopoietic cells. Despite frequent blood transfusion requirement, iron overload (IO) appears to be a rare condition in classic PNH.

OBJECTIVE: To determine the frequency of IO in patients with classic or hemolytic PNH.

PATIENTS AND METHODS: A study in which PNH diagnosis was established measuring by flow cytometry GPI-deficient cells in blood samples of patients with intravascular hemolytic anemia. IO was established as follows: in men, ferritin and transferrin saturation (TS) values >300 ng/mL and >50%, respectively. In women cut-off values for ferritin and TS were >200 ng/mL and >45%, respectively. Along with ferritin, ultrasensitive C-reactive protein (uCRP) was quantitated to rule out concomitant inflammation in all cases. A multiple linear regression model was employed to study the association between ferritin with TS, number of packed red blood cells (PRBC) units transfused and uCRP.

RESULTS: 20 patients with classic PNH, 45% were women, median age 37 years were included. IO was identified in 8 cases (40%). A statistically significant correlation between ferritin with TS and ferritin with number of PRBC units transfused was recorded. In the multivariate analysis, the number of PRBC units transfused showed a highly statistical significant correlation with ferritin ($p<0.0001$).

CONCLUSION: The association between increased ferritin values with PRBC transfusions must alert about the possibility of IO and its consequences in patients with classic PNH.

KEYWORDS: paroxysmal nocturnal hemoglobinuria; iron overload; ferritin; transferrin saturation; blood transfusion

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Sobrecarga de hierro: una complicación pasada por alto en hemoglobinuria paroxística nocturna clásica

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Resumen

ANTECEDENTES: la hemoglobinuria paroxística nocturna, un trastorno adquirido de la médula ósea debido a mutación somática del gen PIG-A, resulta en expresión deficiente de glucosil-fosfatidil-inositol (GPI) y de proteínas ancladas a GPI en la superficie de las células sanguíneas y hematopoyéticas. A pesar de los requerimientos transfusionales frecuentes, la sobrecarga de hierro parece ser infrecuente en la hemoglobinuria paroxística nocturna clásica.

OBJETIVO: determinar la frecuencia de sobrecarga de hierro en pacientes con hemoglobinuria paroxística nocturna clásica o hemolítica.

PACIENTES Y MÉTODOS: estudio en el que el diagnóstico de hemoglobinuria paroxística nocturna se estableció al medir por citometría de flujo las células deficientes en GPI en muestras sanguíneas de pacientes con anemia hemolítica intravascular. La sobrecarga de hierro se estableció como sigue: en hombres, valores de ferritina y saturación de transferrina (ST) >300 ng/mL y >50%, respectivamente. En mujeres las cifras de corte para ferritina y saturación de transferrina fueron >200 ng/mL y >45%, respectivamente. De manera simultánea con la medición de ferritina se cuantificó proteína C reactiva ultrasensible (PCRu) para descartar inflamación concomitante en todos los casos. Se usó un modelo de regresión lineal múltiple para estudiar la asociación entre ferritina con saturación de transferrina, número de unidades de concentrados eritrocitarios transfundidos y PCRu.

RESULTADOS: se incluyeron 20 pacientes con hemoglobinuria paroxística nocturna clásica, 45% mujeres, con mediana de edad de 37 años y en 8 casos (40%) se identificó sobrecarga de hierro. Se encontró una correlación estadísticamente significativa entre ferritina con saturación de transferrina y ferritina con número de concentrados eritrocitarios transfundidos. En el análisis multivariado el número de concentrados eritrocitarios transfundidos mostró una correlación estadísticamente significativa con ferritina ($p>0.0001$).

CONCLUSIÓN: la asociación entre valores elevados de ferritina con transfusión de concentrados eritrocitarios debe alertar acerca de la posibilidad de sobrecarga de hierro y sus consecuencias en pacientes con hemoglobinuria paroxística nocturna clásica.

PALABRAS CLAVE: hemoglobinuria paroxística nocturna, sobrecarga de hierro, ferritina, saturación de transferrina, transfusión sanguínea.

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INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal bone marrow disorder produced by a somatic mutation of the PIG-A gene, which results in poor expression of both glucosil-phosphatidylinositol (GPI) and the proteins anchored to GPI on the surface of hematopoietic cells. PNH is classified as hemolytic or classic, associated with bone marrow failure (either aplastic or with myelodysplasia), and the subclinical variant.[1]

Despite the frequent transfusion requirement associated with PNH, iron overload (IO) appears not to be a frequent condition, particularly in the hemolytic variant. It is well known that packed red blood cell (PRBC) transfusions, in spite of improving quality of life and reducing morbidity and mortality associated with anemia, have deleterious effects.[2,3] There are studies demonstrating that there is a correlation between the number of PRBC transfusions and a decrease in overall survival, due in part to IO, since each PRBC unit contains between 200 mg to 250 mg of iron.[4]

Perhaps hemoglobinuria and hemosidenuria might account for the apparently absence of IO in patients with classic PNH. In the literature there are case reports that describe renal hemosiderosis in association with PNH but not IO.[5-7]

Piperno defined IO as an increase in body iron deposits regardless of end organ damage.[8] IO can be diagnosed by demonstrating an elevated serum ferritin level along with increased serum transferrin saturation (TS). A large screening study of IO in North Americans established that serum ferritin concentrations >300 ng/mL in men and >200 ng/mL in women should raise suspicion of IO.[8,9] The relevance of diagnosing IO lies in the potential benefit of iron chelation therapy in order to prevent organ damage and hence decrease mortality.[10-12] Several studies have shown that transfusion dependency is associated with a lower survival rate.[13-17]

The aim of this study was to determine if there is IO in classic PNH and, if so, to establish its frequency in a group of patients with the disease at a national reference hospital in Mexico City.

PATIENTS AND METHODS

Our hospital admits an important number of cases with so called rare diseases such as PNH. This is a cross-sectional study in a selected group of patients with classic PNH.

Classic PNH diagnosis was made in patients with intravascular hemolytic anemia and the presence of GPI-deficient erythrocytes, granulocytes and/or monocytes identified by flow cytometry in blood samples.[18]

Patients with PNH associated with bone marrow failure, subclinical PNH, malignant neoplasms, autoimmune diseases, active viral hepatitis, abnormal karyotype and positive direct antiglobulin test were excluded from the study.

From each patient with classic PNH, blood samples were drawn in order to measure serum iron, total iron binding capacity, TS, ferritin and lactic dehydrogenase. Since ferritin levels can also increase in states of inflammation, ultrasensitive C reactive protein (uCRP) was concomitantly measured in all patients. A uCRP level >10 mg/dL was consistent with systemic inflammation.

Iron overload was established as follows: in men, ferritin and TS values >300 ng/mL and >50%, respectively. In women cut-off values of ferritin and TS were >200 ng/mL and >45%, respectively.[19]

The number of packed red blood cell (PRBC) units transfused was registered from diagnosis to the date when blood samples were drawn.

Age, gender and time elapsed from diagnosis were also recorded.

We used descriptive statistics, with median and range and mean and standard deviation for continuous variables and frequencies for categorical variables. The association of IO with PNH was tested firstly with a Spearman correlation. In order to test if this association was independent from other variables such as ferritin and number of PRBC units, a linear regression was fitted. We considered a $p<0.05$ to be statistically significant. We used STATA 12 software to analyze the data.

RESULTS

The records from 30 patients with diagnosis of PNH were reviewed. Of these, 10 cases were excluded due to incomplete data or because the diagnosis was PNH other than classic.

Demographic and laboratory data are shown in Table 1. Nine cases were women and 11 men. Their median age was 44 years (range 20 to 68 years) with a median PNH duration of 144 months ranging from 37 to 252 months. Abnormal high values of TS and ferritin were recorded in 11 patients (55%) and 10 cases (50%), respectively. Eight out 20 patients showed increased values of both TS and ferritin. Thus according to IO definition, 40 % of cases in our series have body iron storage in excess.[19]

In all the patients uCRP values were <10 mg/dL, hence ruling out inflammation. The mean number of PRBC units transfused to our patients was 28.9 (range 1 to 118 units, Table 1). Correlation analysis shown in Table 2 revealed that TS with ferritin (r = 0.728; p 0.001), as well as, ferritin with number of PRBC (r = 0.723; p = 0.003) were statistically significant. The multivariate analysis between ferritin with TS, uCRP and number of PRBC showed statistically significant only with transfusion support (Table 3).

Interestingly, a 20 year old male (Patient No. 17), received only one PRBC unit, his uCRP was normal, and both TS and ferritin values were significantly increased. These findings could suggest hereditary hemochromatosis, however no genetic studies were available on him in order to support this diagnosis.

DISCUSSION

It is well known that as chronic intravascular hemolysis is one of the hallmarks of PNH, hemosiderinuria and hemoglobinuria are almost always present, both of which can cause iron deficiency.[1] Because of these, although PNH is a disease in which red blood cell transfusions are commonly needed on a regular basis, IO does not seem to be a frequent complication. To our knowledge there are no reports on this issue besides few case reports of local hemosiderin deposits in the kidneys of patients with PNH.[5,7]

In this study, measuring TS and ferritin in serum, we identified IO in 40% of patients with classic PNH. We focused on the PNH hemolytic group since it is well known that patients with bone marrow failure syndromes may develop IO regardless of PRBC transfusion.[20] The statistically significant correlations recorded between ferritin levels and number of PRBC units, as well as between TS and ferritin values are in line with the notion that PRBC transfusion may account for IO in some patients with classic PNH. Since uCRP values seemingly ruled out inflammation, increased ferritin values support IO.

One of the potential flaws of our study could be the sample size. Bias issues due to small samples in multivariate and regression analysis take place when ten or less subjects for each variable are included.[21] However, in the current study the number of subjects for each variable was twenty far more to that required.

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Table 1. Demographic characteristics, laboratory data and transfusion support in patients with classic paroxysmal nocturnal hemoglobinuria

Patient No.	Gender	Age (yrs)	Transferrin saturation (%)	Ferritin (ng/mL)	Ultrasensitive C-reactive protein (mg/dL)	No. packed red blood cells Units
1	M	33	14.3	24.9	0.349	30
2	M	68	**56.2**	62	1	19
3	M	47	26.2	**662.8**	1.14	22
4	M	51	**97.9**	**3164**	1.59	93
5	M	56	9.1	22.9	0.96	6
6	F	67	12.8	37.4	1.53	23
7	F	36	**63**	**221**	0.87	27
8	F	46	15.6	150	0.5	5
9	M	43	**82.5**	**2010**	0.749	83
10	F	34	19.4	**235**	0.92	17
11	F	33	**55**	**2518**	0.775	118
12	F	58	**89.3**	**957**	1.67	10
13	F	29	**56.5**	**290**	2.1	14
14	F	29	30	126.9	1.45	18
15	M	35	8.6	49.5	0.35	6
16	M	23	34.2	21.6	0.09	12
17	M	20	**87.6**	**1557**	1.16	1
18	F	53	**59.5**	44.4	0.201	21
19	M	37	**94.9**	**3641**	0.371	43
20	M	23	**87**	34.7	0.36	8

Bold numbers indicate abnormal high values.

Table 2. Association between iron parameters and transfusion support

	Age	TS	Ferritin	uCRP
TS	r=0.075 p=0.75	-	-	-
Ferritin	r=0.008 p=0.97	r=0.728 **p=0.001**	-	-
Ultrasensitive C reactive protein	r=0.193 p=0.42	r=0.201 p=0.4	r=0.066 p=0.78	-
No. packed red blood cells	r=0.136 p=0.56	r=0.417 p=0.07	r=0.723 **p=0.003**	r=0.062 p=0.39

Table 3. Multivariate analysis between ferritin with transferrin saturation, ultrasensitive C-reactive protein and transfusion support

Variable	B coefficient	Standard error	p value
Ultrasensitive C reactive protein	40.62	322.94	0.901
No. packed red blood cells	28.41	5.67	<0.0001
Transferrin saturation	16.2	5.64	0.012

R-squared 0.65 for the model.

It is well known that IO can lead to organ damage and hence shorten survival.[22] According to IO guidelines, patients who are dependent on PRBC transfusion, whose life expectancy is >1 year and serum ferritin levels ≥1,000 µg/L are candidates for chelation therapy.[23] Our findings raise the question whether patients with classic PNH and IO would benefit from iron chelation therapy.

In summary, although the number of cases included herein is small the association between PRBC transfusion and ferritin levels appears to be strong. These data should alert about the possibility of IO in patients suffering classic PNH which was identified in 40% of cases. To the best of our knowledge, these findings have not been described before.

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