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Paroxysmal nocturnal hemoglobinuria in México: a 30-year, single institution experience

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ABSTRACT

Background. Paroxysmal nocturnal hemoglobinuria (PNH) stems from chronic, complement-mediated, intravascular hemolysis, which results in anemia, hemoglobinuria, fatigue, and other hemolysis-related disabling symptoms. Novel diagnostic methods have led into an increased identification of the disease. **Aims.** To analyze the salient features of patients with PNH identified in a single institution in México, in a 30-year period. **Material and methods.** The records of 31 patients with PNH identified between 1984 and 2013 were reviewed; 20 females. Median age was 39 years, range 5 to 88. Patients were followed for periods of 0.5 to 221 months, median 46 months. **Results.** Most patients (97%) presented peripheral blood cytopenias, 11 (35%) presented a thrombotic episode, whereas 4 (13%) showed hemolytic anemia. No thrombotic episode was fatal. In the cytopenic group, 4 patients with hemolysis were included and in the patients with the hemolytic variant the red blood cell destruction process was continuous while not paroxysmal. Anemia was recorded in 30 individuals; median hemoglobin levels were 8.5 g/dL, range 3.7 to 12.8. Leukopenia was present in 18 individuals; median white blood cell count was 3.3 x 10^9/L, range 1.6 to 10.8, whereas thrombocytopenia was present in 18 subjects; median platelet count was 67 x 10^9/L, range 6 to 546. Pancytopenia was present in 15 patients. Hemoglobinuria was recorded in 12 patients and low free haptoglobin levels coupled with increased lactic dehydrogenase levels, consonant with hemolysis in 4 patients. **Conclusions.** In México the cytopenic variants are considerably more common than either the hemolytic or the thrombotic variants of the disease, this being particularly relevant since only the hemolytic variants of PNH are the ones which show a good response to the complement-blocking therapy employed nowadays in the treatment of the disease.

Hemoglobinuria paroxística nocturna en México: experiencia de 30 años en una sola institución

RESUMEN

Antecedentes. *La hemoglobinuria paroxística nocturna (HPN) es una enfermedad crónica, mediada por el complemento que causa hemólisis intravascular y se manifiesta con anemia, hemoglobinuria y fatiga. Se ha señalado que la distribución de las distintas formas clínicas de la enfermedad es diferente en México que en otros lugares.* ***Objetivo.*** *Analizar las características de un grupo de pacientes con HPN identificado en un periodo de 30 años en una sola institución privada en México.* ***Material y métodos.*** *Se revisaron los expedientes de 31 pacientes con HPN identificados entre 1983 y 2013. Veinte fueron mujeres y la mediana de edad fue de 39 años, rango de cinco a 88 años. Los pacientes fueron seguidos durante periodos de 0.5 a 221 meses, con una mediana de 46 meses.* ***Resultados.*** *La mayoría de los pacientes (97%) se presentaron con citopenias en sangre periférica, 35% se presentó con un episodio trombótico y 13% se presentó como anemia hemolítica. Ningún evento trombótico fue fatal. En el grupo de pacientes con citopenias se incluyeron los cuatro con hemólisis y en ellos la destrucción de hematíes fue continua y no paroxística. La anemia se registró en 30 individuos, los niveles de hemoglobina promedio fueron de 8.5 g/dL (rango 3.7 a 12.8 g/dL). La leucopenia se encontró en 18 pacientes con una mediana del conteo de glóbulos blancos 3.3 x 10^9/L (rango 1.6 a 10.8^9/L), la trombocitopenia se presentó en 18 sujetos con una mediana en el conteo de plaquetas de 67 x 10^9/L (rango de seis a 546) y la pancitopenia se reportó en 15 pacientes. En 12 pacientes con hemoglobinuria se encontraron valores disminuidos de haptoglobina libre y aumento de los niveles de deshidrogenasa láctica en cuatro pacientes.* ***Conclusiones.*** *La variante citopénica de la HPN*

en México es considerablemente más común que las variantes trombótica o hemolítica de la enfermedad, lo que es particularmente relevante, dado que sólo las variantes hemolíticas son las que muestran una buena respuesta a la terapia inhibidora de la activación del complemento empleada hoy en día como tratamiento de elección para esta enfermedad.

Key words. Paroxysmal. Nocturnal. Hemoglobinuria. PNH. México.

Palabras clave. *Hemoglobinuria. Paroxística. Nocturna. México.*

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a hematological disorder characterized by complement-mediated hemolytic anemia, thrombophilia, and bone marrow failure. PNH stems from a somatic, acquired mutation in the X-linked phosphatidylinositol glycan class A (PIG-A) gene, which impairs the membrane expression on affected blood cells of a number of proteins, including the complement regulators CD55 and CD59.[1] In all cases of PNH, blood cells are deficient not only in CD55 and CD59, but also in a large variety of other membrane proteins that have only one obvious connection to one another: all are anchored to the membrane by a phospholipid, glycosylphosphatidylinositol (GPI). The hallmark of PNH is chronic, complement-mediated, intravascular hemolysis, which results in anemia, hemoglobinuria, fatigue, and other hemolysis-related disabling symptoms. In addition, the peculiar thromboembolic risk typical of PNH patients is thought as secondary to the complement-mediated hemolysis itself and/or to a complement-mediated activation of platelets; in addition, in-travascular hemolysis appears to play also a role in the thrombophilia of the disease, as inhibiting the cytolytic activity of complement markedly reduces the incidence of thromboembolic complications in patients treated with eculizumab.[1]

As a complement-mediated disease, PNH is an appropriate medical condition to develop and to investigate therapeutical complement inhibitors; accordingly, the first complement inhibitor eculizumab, a humanized anti-C5 monoclonal antibody, which has been proven safe and effective for the treatment of PNH patients.[1] Chronic treatment with eculizumab results in sustained control of intravascular hemolysis, leading to hemoglobin stabilization and transfusion independence in more than half of the patients.[1]

PNH was considered a rare disease, but the development of molecular-based treatments has renewed the interest in the condition, its prevalence being re-assesed. Several studies dealing with the prevalence, diagnosis and salient features of the disease in México have been published;[2-18] one of the main findings of these studies is that the hemolytic variant of PNH is rather infrequent, whereas the cytopenic (hypoplastic or dysplastic) variants are the most frequent ones in México.[2,9-10]

We describe here the features of a group of 31 patients with PNH, studied and treated in a single institution, between 1983 and 2013.

MATERIAL AND METHODS

The records of all patients in whom PNH was diagnosed at the Centro de Hematología y Medicina Interna de Puebla in Puebla, México, from January 1983 to August 2013 were reviewed. Between 1983 and 1997 the diagnosis of PNH relied on the abnormality of the classic PNH tests: Ham's test, hemolysis by inulin and hemolysis by sucrose;[2] after 1997 the diagnosis was based on the expression of CD55 and CD59, tested by flow cytometry in red blood cells (RBC), granulocytes and platelets,[12] whereas after 2012, the diagnosis relied on fluoresceinated aerolysin variant (FLAER)-based assays to detect granulocytes and monocytes lacking expression of GPI-linked structures.[19-20]

RESULTS

Patients

Thirty-one patients were identified, 11 males and 20 females. Median age was 39 years, within a range of 5 to 88. Patients were followed for periods of 0.5 to 221 months, median 46 months.

Diagnosis

Fifteen patients were diagnosed employing the classical PNH tests, between 1983 and 1997. Of these, 12 had an abnormal Ham's test, 3 an abnormal inulin test and 8 an abnormal sucrose test, whereas 2

individuals displayed abnormalities in the three tests. Between 1997 and 2013, 16 individuals were diagnosed by assessing the expression of CD55 and CD59 in the cell surface of blood cells, by means of flow cytometry, whereas in 3 patients of the latter group, the diagnosis was confirmed by means of FLAER-based assays.

Laboratory studies

Anemia was recorded in 30 individuals; median hemoglobin levels were 8.5 g/dL, range 3.7 to 12.8. Leukopenia was present in 18 individuals; median white blood cell count was 3.3 x 10^9/L, range 1.6 to 10.8, whereas thrombocytopenia was present in 18 subjects; median platelet count was 67 x 10^9/L, range 6 to 546. Pancytopenia was present in 16 patients. Hemoglobinuria was recorded in 12 patients and low free haptoglobin levels coupled with increased lactic dehydrogenase levels consonant with hemolysis in 4 patients. A bone marrow biopsy was done in 14 patients; hypoplasia was recorded in 9. It was also found that 11 patients had a vaso-occlusive episode (8 in lower limbs, two in cerebral veins and one in mesenteric veins). Five patients had iron deficiency anemia.

Clinical presentation

According to the above mentioned data, most patients (30/ 31 = 97%) presented with peripheral blood cytopenias, 11 (35%) presented with a thrombotic episode whereas 4 (13%) presented hemolytic anemia. Sixteen patients (52%) displayed pancytopenia. In the cytopenic group the 4 patients with hemolysis were included and in the patients with the hemolytic variant the RBC destruction process was continuous and not in paroxysms. Of the 11 patients who had thrombosis, nine had the PNH clone measured and five were found to have a PNH clone above 50%, whereas in four the PNH clone was below 50%. None of the thrombotic episodes was fatal (Table 1).

Treatment and evolution

Of the 31 patients, 26 were given anabolic androgens, 5 were treated with iron salts after recording iron deficiency and two were given an allogeneic bone marrow transplant. No patients were treated with eculizumab. Patients have been followed for a median of 46 months (range 0.5 to 221). Median survival of all the patients has not been reached, whereas the 221-month survival was 74.6%, see figure 1. Two patients have died, one as a result of graft *vs.* host disease 13 months after the diagnosis and 10 months after the allograft, and the other one due to acute renal failure during an hemolytic episode. One patient developed a donor-derived hairy cell leukemia after an allograft but the clinical course has been stable and no treatment has been required.[17]

DISCUSSION

The improvement in the diagnostic capacity of several tests to define the bona fide existence of PNH has resulted in an increase of the identification of cases of this disease.[1,19-21] Flow cytometric analysis of GPI-anchored proteins is the gold standard for diagnosis of PNH. Due to therapy options and the relevance of GPI-deficient clones for prognosis in aplastic anemia detection of PNH is gaining importance. However, no generally accepted standard has been established. PNH diagnosis with flow cytometry traditionally involves the analysis of CD55 and CD59 on RBC and neutrophils. However, the ability to accurately detect PNH RBC is compromised by prior hemolysis and/or transfused RBC; FLAER-based flow assay using CD45, CD33, and CD14 that accurately identified PNH monocyte and neutrophil clones have been described, the FLAER assay on

Table 1. Distribution of the clinical variants of paroxysmal nocturnal hemoglobinuria patients in different places.

	Góngora, *et al.*[9,10]	Ruiz-Argüelles, *et al.*[2]	Hillmen, *et al.*[26]	Socié, *et al.*[27]	This study
Patients (n)	168	13	80	220	31
Site	México	México	England	France	México
Centers	Multicenter	Single center	Single center	Single center	Single center
Cytopenic variant	44%	46%	29%	20%	52%
Hemolytic variant	30%	31%	42%	52%	12%
Thrombotic variant	2.5%	23%	39%	28%	35%

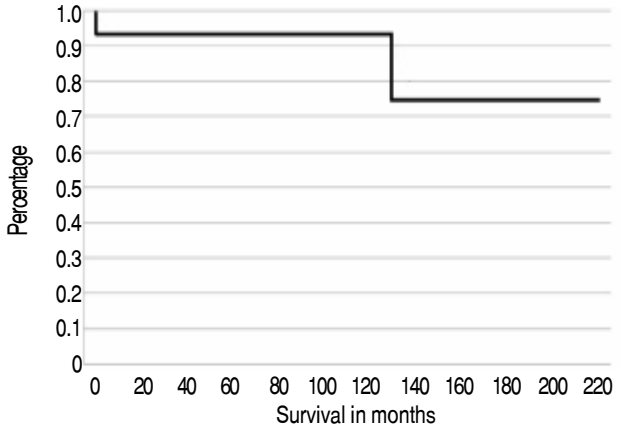

Figure 1. Overall survival of the 31 patients with paroxymal nocturnal hemoglobinuria.

white blood cells being the most sensitive and robust primary screening assay for detecting PNH clones in clinical samples.[22] The use of updated methods to define the existence of PNH has resulted in an increase of incidence of the disease: In a 30-year period (1950-1980) in a large hospital in México City which concentrates cases of hematological diseases from all over the country, only 13 cases of PNH were diagnosed, employing the classical tests of Ham, inulin and sucrose,[2] whereas in a similar period (1984-2013), we have now identified 31 cases of PNH in a smaller, private-practice institution.

The flow cytometric definition of PNH has totally replaced the use of the traditional PNH tests: Ham's test, hemolysis by inulin and hemolysis by sucrose.[21] Some concerns about over-diagnosing PNH with these new methods has been raised[12] and it has been shown in our country that around 50% of patients with histologically proven bone marrow hypoplasia do show PNH clones in the peripheral blood and/or in the bone marrow.[12] There has also been some observations about a different distribution of the clinical variants of PNH in Mexican mestizos as compared with other populations. Specifically, it has been described that the cytopenic variants of PNH in México (both hypoplastic and dysplastic) are considerably more common than either the hemolytic or the thrombotic variants of the disease,[9-10] this being particularly relevant since only the hemolytic variants of PNH are the ones which show a good response to the complement-blocking therapy employed nowadays in the treatment of the disease.[1,23] The data which we are presenting here support these previous observations, since we have found that the cytopenic variants of PNH were the most frequent ones, followed by the thrombotic variant, the hemolytic form being the less frequent one (only 13% of all the PNH cases). The increased prevalence of the cytopenic variant of PNH in México may stem from the high prevalence of aplastic anemia in México,[24,25] since most hematologists in México look for PNH clones in patients with bone marrow hypoplasia, a condition in which PNH clones have shown to be present in up to 50% of patients.[12] We have also found that the hemolytic variant is lower than that reported from Caucasian populations[26–29] and that the thrombotic variant is about the same as that informed from Caucasians (Table 1). As compared with previous information published from México, our increased incidence of the thrombotic variant of PNH may stem from our long-standing interest in thrombophilic conditions;[30,31] this factor may introduce bias in the increased identification of this variant. Interestingly, we were unable to find a correlation between the size of the PNH clone and the prevalence of thrombotic episodes *(vide supra)*.

CONCLUSION

We have found that PNH has been underdiagnosed in México and that by using modern methods more cases are being and will be identified. The clinical presentation of the disease in México has some peculiarities since hemoglobinuria presented only in a fraction of the patients and was not paroxysmal neither nocturnal. The cytopenic variant of the disease was the most prevalent in México than in other populations and the hemolytic variant was found to be the less frequent one. More studies are needed to describe the true incidence of the disease employing the new methodology and diagnostic criteria.

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Recibido el 29 de agosto 2013.
Aceptado el 10 de diciembre 2013.