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Gasser cell: A biomarker of response to enzyme replacement therapy in patients with mucopolysaccharidosis type VI

Célula de Gasser: Um biomarcador de resposta à terapia de reposição enzimática em pacientes com mucopolissacaridose tipo VI

Célula de Gasser: Un biomarcador de respuesta a la terapia de reemplazo enzimático en pacientes con mucopolisacaridosis tipo VI

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Abstract
The mucopolysaccharidosis (MPS) type VI is a rare lysosomal storage disease presenting leukocyte inclusions (Alder-Reilly anomaly) and lymphocytes with metachromatic inclusion surrounded by clear spaces, Gasser cells. Currently, an enzyme replacement therapy (ERT) with galsulfase is used to treat MPS type VI. This study evaluated 14 patients with MPS type VI performed cell counts Gasser before and after six months from the beginning of ERT. It was observed an average of 12.7% cells per patient, and after six months was found complete cell Gasser disappearance, proving to be an effective biomarker of response to ERT.
Keywords: Enzyme replacement therapy; Galsulfase; Leukocyte inclusions; Lysosomal storage disease; Mucopolysaccharidosis type VI.

Resumo
A mucopolissacaridose (MPS) tipo VI é uma doença rara de armazenamento lisossomal que apresenta inclusões leucocitárias (anomalia de Alder-Reilly) e linfócitos com inclusões metacromáticas circundada por espaços claros,

chamadas de células de Gasser. Atualmente, uma terapia de reposição enzimática (TRE) com galsulfase é usada para tratar a MPS tipo VI. Este estudo avaliou 14 pacientes com MPS tipo VI realizando contagem de células de Gasser antes e após 6 meses do início da TRE. Observou-se em média 12,7% de células por paciente, e após 6 meses foi constatado o desaparecimento completo das células de Gasser, demonstrando ser um biomarcador eficaz de resposta à TRE.
Palavras-chave: Doença de armazenamento lisossomal; Galsulfase; Inclusões de leucócitos; Mucopolissacaridose tipo VI; Terapia de reposição enzimática.

Resumen
La mucopolisacaridosis (MPS) tipo VI es una enfermedad rara de almacenamiento lisosómico que presenta inclusiones leucocitarias (anomalía de Alder-Reilly) y linfocitos con inclusiones metacromáticas rodeadas de espacios claros, denominados células de Gasser. Actualmente, se usa una terapia de reemplazo enzimático (TRE) con galsulfasa para tratar MPS tipo VI. Este estudio evaluó a 14 pacientes con MPS tipo VI realizando un recuento de células de Gasser antes y después de 6 meses desde el inicio de la TRE. En promedio, se observó un 12,7% de células por paciente, y a los 6 meses se encontró la desaparición completa de las células de Gasser, demostrando ser un biomarcador eficaz de respuesta a la TRE.
Palabras clave: Enfermedad por almacenamiento lisossômico; Inclusiones de leucócitos; Galsulfasa; Mucopolisacaridosis tipo VI; Terapia de reemplazo enzimático.

1. Introduction

Mucopolysaccharidosis (MPS) VI, or Maroteaux-Lamy syndrome, is a rare autosomal recessive lysosomal storage disease characterized by rapidly progressing systemic manifestations (Khan et al., 2017). Studies conducted in 14 countries of 5 continents show an average prevalence of the disease ranging from 1 in 43,261 to 1 in 1,505,160 live births (Valayannopoulos et al., 2010). Among the different MPS types, the frequency of MPS VI is 2 to 4% in Scandinavia, 3% in Holland, 16% in Portugal, and 18.5% in Brazil (Valayannopoulos et al., 2010). MPS VI is caused by reduced or absent activity of the enzyme N-acetylgalactosamine-4-sulfatase (arylsulfatase B) responsible for the degradation of dermatan sulfate, a glycosaminoglycan (GAG). This deficiency leads to the accumulation of this complex carbohydrate inside cells, tissues, and organs, causing cell dysfunction and severe organ damage (Giugliani et al., 2007). The MPS disorders are classified into seven different types, but only MPS VI shows leukocyte inclusions (Alder-Reilly anomaly). Lymphocytes with these inclusion bodies are called Gasser cells and are characterized by metachromatic inclusions surrounded by a clear space (Heron et al., 2004; Fenneteau et al., 2009).

More than 130 mutations that result in the reduced or absent activity of N-acetylgalactosamine and consequent inhibition of dermatan sulfate degradation are currently known (Tomanin et al., 2018; Valayannopoulos et al., 2010; Villani et al., 2010). MPS VI can show a rapid or slow progression. The onset of symptoms occurs before the second or third year of life in patients with rapidly progressing disease, and mobility is compromised by age 10. Puberty might be absent or delayed, and there are reports of death due to heart failure in the second or third decade of life if the disease progresses rapidly. The late onset of symptoms characterizes the slowly progressing form. The symptoms are milder and may not appear early as seen in the rapidly progressing form but are usually observed in adolescence or early adulthood (Valayannopoulos et al., 2010).

The symptoms seen in patients with MPS VI include facial infiltration, hepatosplenomegaly, growth reduction, joint contractures, cardiovascular changes, ocular abnormalities (corneal opacity, glaucoma, and optic atrophy), neurological abnormalities (hydrocephalus, spinal cord compression, mental impairment is uncommon), obstructive sleep apnea, umbilical and inguinal hernias, and osteoarticular manifestations (Honjo et al., 2020; Harmatz & Shediac, 2017). The Alder-Reilly anomaly (Gasser cells) is only observed in the disease's rapidly progressing form (Cardoso-Santos et al., 2008).

Laboratory screening for all MPS forms is made based on the detection of GAGs in urine by the 1,9-dimethylmethylene blue (DMMB) method. In most cases, the diagnosis is made by detecting reduced or absent enzyme activity in leukocytes. Fibroblast cultures are less frequently used for enzyme assays. The diagnosis can be confirmed in some

cases by genetic analysis (Neufeld et al., 2001).

Until 2005, the only treatment for MPS VI was bone marrow transplantation. Enzyme replacement therapy (ERT) with recombinant human arylsulfatase B (rhASB, galsulfase), genetically engineered from Chinese hamster ovary cells, is currently available (Harmatz et al., 2019; Concolino et al., 2018). The 6-minute walk test and urinary GAG levels are used as biomarkers of MPS VI (Khan et al., 2018). In addition, GAG is a good marker of disease severity, as Swiedler et al (2005) reported. However, urinary GAG has some of the same problem low dose of 0,2 mg/kg gives a reasonably no functional response (Harmatz et al., 2008; Turbeville et al., 2011). The 6-minute walk test is not a reliable biomarker of treatment response since it is susceptible to external conditions that are not related to removing the substrate (Valayannopoulos et al., 2010). Heparin cofactor II-thrombin complex (HCII-T) and dermatan sulfate-chondroitin sulfate ratio are biomarkers of ERT's short-term and long-term effects in patients with MPS types I, II, and VI and are therefore not specific for patients with MPS VI (Langford-Smith et al., 2011).

2. Methodology

A longitudinal study was carried out with MPS VI patients (Hochman et al., 2005). 14 patients with MPS VI were evaluated before and after six months of treatment with rhASB. All patients were diagnosed with deficient ASB activity in leukocytes and/or fibroblasts. The patients were treated from August 2014 to October 2018 at the Treatment Center for Inborn Errors of Metabolism, Barão de Lucena Hospital, Recife, Pernambuco, Brazil, a reference center of enzyme replacement therapy. Gasser cell counts were obtained before and after six months of enzyme replacement therapy. For this purpose, 5 mL peripheral blood was collected into a vacuum tube containing EDTA as an anticoagulant. The blood smear (slide) was prepared at the time of blood collection, and the slide was stained with May-Grünwald and Wright stains. Gasser cells were counted in a Beckman Coulter Gen-S hematology analyzer at the Marcelo Magalhães Laboratory, Recife, Pernambuco, Brazil. After the first blood collection, all patients received infusion treatment of the deficient enzyme (1 g/kg body weight) once a week for at least 4 hours.

Before and after enzyme replacement therapy, Gasser cell counts were calculated as the percentage of these cells in 100 lymphocytes counted in one or more peripheral blood smears by light microscopy at 100x magnification under oil immersion.

3. Results and Discussion

The average age of the 14 patients before enzyme replacement therapy was 8.5 years (range: 1 to 32 years). There were five male and nine female patients. The clinical features observed in MPS VI varied among the 14 patients before ERT, hepatosplenomegaly was observed in 50% of the patients (n = 7), corneal clouding in 42.8% (n = 6), umbilical and inguinal hernias in 28.5% (n = 4) and short stature in 92.8% (n = 13). In contrast, coarse facial features, skeletal deformities, and cardiovascular disease, to a variable extent, were observed in all patients.

Gasser cells were detected in all patients before enzyme replacement therapy, with an average percentage of these cells of 12.7%. The Alder-Reilly anomaly was also identified in all patients studied. In Table 1 it is possible to see the date of the first treatment for which patient and the percentage of Gasser cells before the treatment. Gasser cells' complete clearance was seen in all patients after six months of treatment (Table 1). However, Alder-Reilly inclusions could still be detected in neutrophils, monocytes, eosinophils, and basophils.

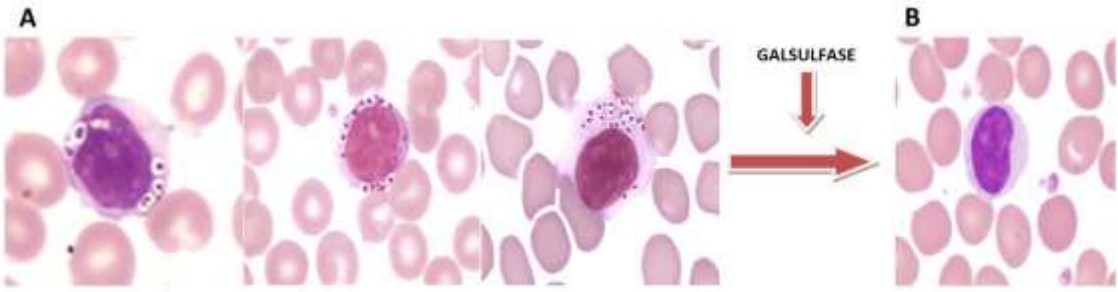
Table 1 – Gasser cell count in patients with mucopolysaccharidosis type VI before and after 6 months of enzyme replacement therapy.

Patient	Age (years)	Date of the first treatment	Gasser cell count before treatment (%)	Gasser cell count after treatment (%)
1	2	21/08/2014	31	0
2	6	18/08/2015	9	0
3	6	13/08/2015	5	0
4	13	18/08/2015	3	0
5	13	03/09/2015	11	0
6	9	04/09/2015	16	0
7	15	05/09/2015	10	0
8	5	04/01/2016	11	0
9	4	04/11/2016	2	0
10	32	04/11/2016	6	0
11	3	24/02/2016	9	0
12	1	03/03/2017	25	0
13	10	20/10/2017	12	0
14	2	28/06/2018	28	0

Source: Authors (2021).

In Figure 1A, it is possible to see lymphocytes of patients with MPS type VI with characteristic cytoplasmatic inclusions called Gasser cells. After 6 months with enzyme replacement therapy, the inclusions were absent (Figure 1).

Figure 1 – Blood smear of a patient with mucopolysaccharidosis type VI. A) Lymphocytes before enzyme replacement therapy - Gasser cells; B) Normal lymphocyte after six months of treatment.



Source: Authors (2021).

Biomarkers used to assess the response to ERT in MPS VI have been reported in the literature (Kubaski et al., 2020; Aerts et al., 2008). However, further studies are needed to identify more effective biomarkers of ERT response. This study showed an association between enzyme replacement therapy with galsulfase for MPS type VI and Gasser cells' clearance six months after the treatment. The results suggest using a less costly and less invasive biomarker to evaluate enzyme replacement therapy's treatment effects than other available methods, such as a 6-minute walk test, urinary GAGs, and ASB activity. Other recently described biomarkers are heparin cofactor II-thrombin complex and the dermatan sulfate-chondroitin sulfate ratio.

HCII-T and dermatan sulfate-chondroitin sulfate ratio are biomarkers of ERT's short-term and long-term effects in patients with MPS I, II, and VI (Kubaski et al., 2020). A 25-fold increase of HCII-T levels is observed in patients who accumulate dermatan sulfate and an increase of approximately 4-fold in patients accumulating heparan sulfate. HCII-T is therefore not a specific biomarker of the response to ERT in patients with MPS VI. Elevated levels are observed in patients

who store dermatan sulfate, i.e., those with MPS I, II, VI, and VII, and those who store heparan sulfate, i.e., patients with MPS III. However, MPS VII is rare, and patients' samples were not available to test the hypothesis (Lanford-Smith et al., 2011).

This study represents a comprehensive analysis of biomarkers of the response to ERT in patients with MPS VI. Gasser cell count was a robust biomarker of the laboratory response to ERT in the 14 patients studied. Urinary excretion of GAGs (dermatan sulfate) is the only biomarker used to monitor the response to ERT that is widely accepted for the evaluation of GAG deposits. Also, it is a promising biomarker for assessing disease severity, as demonstrated in the survey of Swiedler et al (2005). However, urinary GAGs present a low dose of 0,2 mg/kg, and GAG levels decline with age. The time necessary to evaluate the recombinant enzyme's effect has been suggested to be 24 weeks, as indicated in the package insert of galsulfase (Harmatz et al., 2008). The enzyme's effect was evaluated 24 weeks after the beginning of ERT by the 6-minute walk test.

4. Conclusion

In conclusion, Gasser cell count before and after ERT is a valuable biomarker for confirming the diagnosis and assessing therapy's response. This study showed that Gasser cells are found in the rapidly progressing form of MPS type VI. These cells can be used as a biomarker for assessing the response to ERT with galsulfase. However, further studies are needed to evaluate the time necessary for the complete removal of these cells from peripheral blood after the beginning of therapy. Therefore, cell counts should be performed in the first, second, and fourth weeks and the third month after the first treatment.

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