ANÁLISE CLÍNICA E MUTACIONAL DE UMA COORTE DE PACIENTES PORTUGUESES COM PARAGANGLIOMAS DO CORPO CAROTÍDEO

CLINICAL AND MUTATIONAL ANALYSIS OF A COHORT OF PORTUGUESE PATIENTS WITH PARAGANGLIOMAS OF THE CAROTID BODY

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RESUMO

Introdução: Representando 0,03% de todos os tumores, os paragangliomas (PGLs) são extremamente raros. Nos últimos 15 anos, houve um progresso significativo no conhecimento da genética dos PGLs. Mutações germinais nos genes da succinato desidrogenase (SDH) são a causa genética mais comum de PGL.

Objetivo: Dada a elevada relevância clínica do gene da SDH, pretendemos avaliar o valor diagnóstico e prognóstico das mutações de SDH numa coorte portuguesa com paragangliomas do corpo carotídeo.
Descrevemos ainda a experiência de diversos departamentos de Angiologia e Cirurgia Vascular no tratamento destes tumores.

Métodos: Quarenta e seis indivíduos foram incluídos no presente estudo, dos quais quarenta e dois eram casos-índice e quatro eram familiares. Características clínicas e dados bioquímicos foram obtidos através de uma análise retrospectiva de arquivos clínicos. O ADN foi isolado a partir de amostras de sangue periférico obtidas de todos os indivíduos após o seu consentimento informado por escrito. Para todos os indivíduos recrutados, a análise genética envolveu a pesquisa de mutação de toda a região codificadora do gene SDHD que foi estendida ao gene SDHB, em um subgrupo de pacientes com apresentação mais agressiva.

Resultados: Desde 2016 até o momento, a análise genética foi oferecida a 46 pacientes com PGL do corpo carotídeo. Os pacientes têm origem em departamentos de Angiologia e Cirurgia Vascular de todo o país.
A análise genética identificou sete mutações heterozigoticas diferentes no gene SDHD (p.Met1Val, p.Met1Ile, p.Gly12Ser, p.Pro53Leu, IVS3 + 4G> A, IVS3-2A> C, p.Leu139Phefs). Além disso, uma mutação no gene SDHB (p.Ser198Alafs) também estava presente em um caso índice. As mutações do SDHD foram identificadas em oito dos 41 pacientes-índice (20%) e em três dos quatro casos familiares estudados (75%). Destes, uma mutação, p.Met1Ile, estava presente em dois pacientes aparentemente não relacionados.

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A prevalência do tipo familiar variou entre os estudos relatados de 5% a 30%, em nosso estudo, eles representam 26% (12 casos) da amostra. Essa variabilidade ampla resulta da existência de casos familiares ocultos.

Conclusão: O screeninggenético permite a identificação de casos familiares e melhora a tomada de decisão clínica dos pacientes e seus familiares. O presente estudo contribui para uma caracterização mais ampla do perfil molecular de pacientes europeus com PGL.

Palavras-chave
Paragangliomas; Mutações SDH; Cirurgia

ABSTRACT

Introduction: *Representing 0.03% of all tumors, paragangliomas (PGLs) are extremely rare. During the past 15 years, there has been a significant progress in the knowledge of the genetics of PGLs. Germline mutations in succinate dehydrogenase (SDH) genes are the commonest genetic cause of PGL.*

Objective: *Given the high clinical relevance of the SDH status, we aimed to evaluate the diagnostic and prognostic value of SDH mutations in a Portuguese cohort with carotid body paragangliomas.*
We Report the experience of several departments of Angiology and Vascular Surgery in the management of carotid body tumors.

Methods: *Forty-six individuals were included in the present study, of which forty-two were index cases and four were familial cases. Clinical features and biochemical data were retrieved by a retrospective analysis of clinical files. DNA was isolated from peripheral blood samples obtained from all individuals following their written informed consent. For all the subjects recruited, the genetic analysis involved the mutation search on the entire coding region of the SDHD gene and was extended to the SDHB gene coding region, in a subgroup of patients with a more aggressive presentation.*

Results: *Since 2016, genetic screening was offered to 46 patients with Carotid body PGL. The patients came from Angiology and Vascular Surgery Departments from all over the country.*
The genetic analysis identified seven different heterozygous mutations in the SDHD gene (p.Met1Val, p.Met1Ile, p.Gly12Ser, p.Pro53Leu, IVS3+4G>A, IVS3-2A>C, p.Leu139Phefs)In addition, one mutation in SDHB gene (p.Ser198Alafs) was present in one index case. The SDHD mutations were identified in eight of the 41 index patients (20%), and in three of the four familial cases studied (75%). Of these, one mutation, p.Met1Ile, was present in two apparently unrelated patients.
The prevalence of the Familial type has varied between reported studies from as low as 5% to as high as 30%, in our study they represent 26% (12 cases) of the sample. This wide-ranging variability stems from the existence of hidden familial cases.

Conclusion: *Genetic screening allows the identification of familial cases and improves clinical decision-making and adequate management of patients and their relatives. The Present data contributes to a broader characterization of the molecular profile of European patients with PGL.*

Keywords
Paragangliomas; SDH mutations; Surgery

INTRODUCTION

PGLs are extremely rare, the incidence is less than 1 in 30000. Even though typically benign and curable by surgical resection, up to 6% can be malignant[1]. Scoring systems based on morphology and clinical features to assess malignant potential have been proposed but have yet to be fully validated[2]. Approximately 50% occur in the head and neck region, most frequently as hypervascularized carotid body. Patients may thus notice a slow growing and painless lateral neck mass, pulse-like sensations or voice changes[3].
Carotid Body PGLs have been traditionally subdivided in three different types: Sporadic, Familial and Hyperplastic. Representing around 85% of Carotid Body PGLs, the Sporadic form is the most common type.

To this day, three genes associated with Familial PGLs have been identified. All three encode subunits (D, B and C) of the enzyme succinate dehydrogenase complex (SDH), which plays a pivotal role in both the Kreb's cycle and electron transport chain. It has been postulated that a defective succinate dehydrogenase can cause an intracellular increase of vascular endothelial growth factor (VEGF) and hypoxic molecular mediators, leading to hyperplasia, angiogenesis and neoplasia[4].
It's estimated that up to 70% of familial PGL cases carry germline mutations in one of the three SDH genes. In reality, it's likely that this percentage is even higher because most studies don't test for large deletions.
Surgical resection is recommended for most CBTs in healthy patients because of the risk of local complications related to tumor size and a small but definite risk of malignancy. The surgical technique has remained unchanged throughout the years. The most common complication associated with surgical resection is cranial nerve injury which can occur in 19% to 49% of patients. The larger and more adherent the tumor, the higher the risk of cranial nerve injury. Fortunately most cranial nerve deficits are usually temporary[5].

METHODS

Forty six patients diagnosed with CBT in the Angiology and Vascular Surgery Departments of 9 different hospitals in Portugal between 1989 and 2017, were retrospectively evaluated. All patients underwent ultrasound, magnetic resonance or computerized angiotomography imaging preoperatively and the confirmation of the diagnosis of CBTs was made by histopathologic examination after surgical resection. All patients had unilateral tumors except for two who had bilateral carotid tumors. Data on demographics, imaging diagnostic, family history, clinical presentation, surgical treatment, complications, and the patients outcome were retrieved from the clinical files. In addition, the tumour classification according to the Shamblin type was also recorded. For the present study, all these identified patients were contacted and, after their informed consent, a blood sample was taken for genetic testing on SDHx genes.

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) 22. X2 test was used to assess differences between categorical variables. Normality of continuous variables was first assessed by the Kolmogorov-Smirnov test and statistical differences between groups were analyzed either through the t-Student test or the Mann-Whitney U test.

RESULTS

A total of forty six patients 21 male and 25 female ; mean age of 53,1±16,6 years (15-84) were retrospectively evaluated. There were no differences in gender or age of onset between patients carrying SDHx mutations and non-mutation carriers.
The genetic analysis identified seven different heterozygous mutations in SDHD gene in eleven patients (24%). Of these, four were missense mutations, previously reported in Paraganglioma patients, (NP002993.1:p.Met1Val, p.Met1Ile, p.Gly12Ser, p.Pro53Leu), one was a novel frameshift mutation, (NP002993.1:p.Leu139Phefs*29), not previously identified and two were splicing mutations (NM_003002.3:c.314+5G>A and c.315-2A>C), one of which was also a novel mutation, as presented in TABLE 2. The mutation search on SDHB gene revealed a frameshift mutation in one patient (NM_003000.2:c.591delC; NP_002991.2:p.Ser198Alafs*22) previously associated with the Paraganglioma phenotype.
Preoperative embolization of the tumour was performed in six patients. The choice for this technique was due to the size of the tumor, two patients had a Shamblin II and four had a Shamblin III tumor. No strokes or other major complications occurred after preoperative embolization with polyvinyl alcohol particles 1 day before surgery.
The incidence of neurological complications was 8.7 % for TIA and 6.5 % for stroke.
The incidence of cranial nerve injury (especially the Hypoglossal nerve) was 21.71%. One of the patients developed a Horner's syndrome after resection of the tumor. Patients with larger tumors had a higher lesion rate.
Vascular resection and reconstruction was performed in 5 tumors (10.4%).

DISCUSSION

For the present study, the 46 identified CBT patients were contacted and after their informed consent, they underwent genetic testing for SDHD gene, since mutations in this gene are the leading cause of HNPGLs worldwide. The genetic analysis revealed seven different heterozygous variants in SDHD gene in eleven patients with CBT, of whom eight had a positive family history. These variants consisted of four missense mutations, one frameshift and two splicing mutations, of which, two were not reported previously. The prevalence of the familial CBT cases varies between reported studies from as low as 5% to as high as 30%, in our study they represent 8/46 (17%)

of the sample. As expected, the percentage of cases with positive family history was significantly higher in patients with SDHx mutations than in patients with no mutations identified (p < 0.001).
In contrast to previous studies that reported the occurrence of SDHx mutant tumors at a younger age, in the present study there was no difference in age at diagnosis between patients carrying SDHx mutations and non-mutation carriers. This discrepancy might be explained in part by the fact that the mutation analysis for SDHB gene was not performed for all the individuals.
The rate of complications varies greatly from one study to another. In our study the incidence was 8.7 % for TIA and 6.5 % for stroke. Stroke occurred in two patients with tumors classified as Shamblin III and one as Shamblin II. Only one patient had a mutation in SDHD. Despite the complexity of the tumors, 2/3 were Shamblin III with an average diameter of 48 mm, we presented a high morbidity.
Vascular resection and reconstruction was performed in 5 tumors (10.4%) and 2 of these patients had a stroke. These complications may be related to the clamping time which in these patients is less well tolerated. In all cases a great saphenous vein was harvested from the leg to be used as a conduit. Early detection of smaller tumors should reduce the morbidity of the procedure.
Carotid body PGLs are mostly benign, slow-growing tumors. Only 6 % of carotid body disclose uncertain malignant potential. Therefore, performing genetic tests in all CBT patients may be an interesting strategy so that only those with a higher risk of metastization can be selected for surgery. The watchful waiting approach is an option that is currently used in bilateral tumors because of the risk of baroreceptor reflex syndrome and may be an option in patients with unilateral paragangliomas and a low risk of malignancy.
The establishment of a genotype / phenotype relationship may allow to select the best candidates for this strategy and thereby reduce the morbidity in this disease[6].

In conclusion, genetic screening allows identifying familial cases and improves clinical decision-making and adequate management of patients and their relatives. The present data contributes to a broader characterization of the clinical and molecular profile of European patients with CBT

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