ABSTRACT

Objective: To describe the clinical data and the results of molecular analyses of the mitochondrial DNA in a patient with Kearns-Sayre Syndrome.

Methods: Molecular analyses of mitochondrial DNA from the patient included PCR amplification of a region where the common Kearns-Sayre deletion is located and Genotype-Phenotype correlations are discussed.

Results: The affected patient showed ptosis, progressive external ophthalmoplegia, pigmentary changes in the peripheral retina and right bundle block. Molecular analysis disclosed a ~5kb deletion in the mitochondrial DNA and some wild type mtDNA indicating heteroplasmy.

Conclusions: Molecular analysis of mitochondrial DNA confirmed the clinical diagnosis of Kearns-Sayre syndrome. PCR provides a rapid method to identify the common 4997 bp deletion in Kearns-Sayre Syndrome.
Sayre syndrome. In such cases, PCR diagnosis could avoid invasive methods such as muscle biopsy or spinal tap (Arch Soc Esp Oftalmol 2008; 83: 155-160).

**Key words:** Kearns-Sayre syndrome, strabismus, ophthalmoplegia, mitochondrial DNA, heteroplasmy.

**INTRODUCTION**

Mitochondrial cytopathies are an uncommon group of multi-systemic diseases exhibiting biochemical, histopathological and/or genetic evidence of mitochondrial dysfunction (1). These diseases express as well-defined clinical syndromes and are caused by molecular defects in mitochondrial DNA (mtDNA) which may vary from one-off mutations to larger genic rearrangements such as duplications or deletions (2). Tissues with large metabolic demand of oxidative energy, such as muscles and the brain, are particularly vulnerable to mtDNA mutations even though the diseases related to it can be differentiated on the basis of their clinical characteristics. Most of them share the expression of lactic acidosis and massive proliferation of mitochondria in the muscle which gives rise to a typical histopathological pattern of torn-red fibers (3). The clinical expressions generally begin in childhood and can include lactic acidosis, anemia, myopathy, neurological abnormalities, endocrine alterations, kidney diseases, neurosensory deafness, non-typic retinal dystrophy and defects in the heart conduction system, the latter being the main cause of premature death.

The three main diseases associated to mtDNA deletions are Pearson’s syndrome, chronic progressive external ophthalmoplegia and Kearns-Sayre syndrome (SKS, OMIM #530000) (4). The latter is a pleiotrophic disorder first described in 1958 and characterized by chronic progressive external ophthalmoplegy, pigmentary retinopathy, cardiac obstructions and cerebral ataxia (5).

The usual age of onset is before 20 years of age. Affected subjects frequently exhibit associated clinical data such as deafness and high concentration of proteins in the cerebro-spinal liquid (6). Other frequent endocrine symptoms are diabetes, hyperaldosteronism and amenorrhea.

The mtDNA deletions associated to SKS are variable and range from 1.3 to 8 kilobases (kb) with different amounts of deleted mtDNA present in different tissues, which correlates with the multi-systemic nature of the syndrome (7-9). The most frequent rearrangement observed in the mtDNA of patients with SKS is «common deletion » of 4977pb (mtDNA 4977), localized between nucleotides 8482 and 13640 (10) of mtDNA and which is found in approximately 50% of affected subjects (10). In addition to said mtDNA deletions, specific mutations have also been identified in some SKS patients (6).

There are few publications describing a complete ophthalmological research of SKS patients as well as mtDNA molecular analysis. This paper presents the results of a full ophthalmological evaluation as well as the mtDNA analysis of a Mexican patient with SKS diagnostic by PCR technique which allows for the identification of common deletion in mtDNA of SKS patients either in heteroplasmic or homoplasmic state.

**SUBJECTS, MATERIAL AND METHODS**

A 15 year-old patient was referred to our hospital due to ptosis and limitation of bilateral eye move-
ments dating 2 years back. The patient did not refer history of diabetes, high pressure or trauma, although she had a gynecological history of menarche at age 13 with regular periods. No family history of ptosis, myopathies or hereditary disease was found. Upon physical exploration, the height of the patient was 160 cm and the neurological exploration did not evidence muscular dystrophy, ataxia or deafness. The ophthalmological exploration revealed a visual acuity in the right eye (RE) of 20/40 and in the left eye (LE) of 20/25; the IOP was of 11 mmHg (RE) and 12 mmHg (LE). Eye movements exploration showed a marked limitation in all gaze directions for both eyes, with an exotrophy of 15 prismatic dioptries (fig. 1). Palpebral opening was of 7 mm in both eyes, with highly reduced movement of the elevator in both eyes (5 mm) and 1 mm margin pupillary reflex in both eyes. The pupil diameter was within normal ranges and pupil reflexes were intact. No alterations were found in the anterior segment of both eyes. Eye fundus exploration under pupil dilatation revealed multiple pigmentary changes with a «salt and pepper» pattern along the retinal equator and periphery of both eyes (fig. 2). Fluorescein angiography (FA) showed moderate atrophy of the retinal pigmented epithelium (fig. 3), while the electroretinograph evidenced a reduction of the response threshold to light stimuli with previous dilatation and adaptation to darkness. Cytological electromyography showed involvement of all extraocular muscles, the frontal muscle and the pharyngeal muscles. The 12-derivation electrocardiography revealed incomplete obstruction of the right branch of His’s bundle.

The cytological and chemical analysis of the spinal-cerebral fluid obtained with a lumbar punction showed high protein levels (124 mg/dl). The patient was clinically diagnosed with the Kearns-Sayre syndrome and referred to the Genetic Department.

Molecular studies of mitochondrial DNA: the investigation was made with the authorization of the Bioethics Committee of the institution and informed consent signed by the patient’s parents. On the basis of an 8-ml sample of peripheral blood, a platelet concentrate was obtained from which the mt DNA was isolated with the help of the QIAamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer indications. The mtDNA was amplified with PCR utilizing MT-1 and MT-4 primers indicated in table 1, taken from Brenner et al (13). MT-1 borders the “common deletion” mtDNA region of SKS in the 5’ direction, while MT-4 borders the deletion site in the 3’ direction. This pair of primers amplifies a product having ~5kb: if the «common deletion» of 4977pb of the SKS is present, the obtained PCR product will be of 550 pb. In the case that the deletion is absent, the expected ~5kb will be amplified. The heteroplasmic mtDNA will exhibit both ranges. Every 25 µl of PCR reaction included 150 ngs of mtDNA, MgCl 2 at a final concentration of 1.5 mM, 200 µM of each

Fig. 1: Bilateral ptosis and ophthalmoplegy in a 15 year-old patients with Kearns-Sayre syndrome diagnostic.

Fig. 2: Appearance of the posterior pole showing pigmentary changes and pallor in the optic nerve head.

Fig. 3: Fluorescein angiography showing diffuse atrophy of the retina pigmented epithelium.
dNTP, 1 µM of each primer, and 1.25 U of HotStart Taq polymerase (Quiagen). Primers MT-1 and MT-4 were utilized and the temperature program included an initial denaturation cycle at 95°C for 15 minutes, 40 denaturation cycles at 95°C for 1 minute, alignment at 65.2°C per minute and extension at 72°C for 3 minutes, with a final extension cycle at 72°C for 10 minutes.

A standard 100 pb DNA marker was used to confirm the size of the amplified products. To discard any possibility of contamination caused by PCR amplification, each PCR reaction included a tube in which the DNA template was omitted and substituted by H2O which served as negative control.

RESULTS

The PCR amplification of the patient’s mtDNA with SKS revealed the presence of two products: a 550 range which indicated the presence of the “common deletion” of ~5 kb and a range of wild mtDNA (fig. 4, line 3), which proved the presence of mitochondria with normal DNA and others with DNA which included the deletion (heteroplasmia). In contrast, PCR amplification in the mtDNA of a normal subject only evidenced a ~5 kb range, excluding the presence of deletion (fig. 4, line 2). These results were consistent after 3 independent mtDNA amplifications of the patient and a positive control. In none of the cases the negative control gave mtDNA amplification, excluding amplification due to contamination with DNA.

DISCUSSION

SKS is an infrequent neuromuscular disease characterized by its onset before age 20, chronic progressive external ophthalmoplegy and pigmentary degeneration of the retina. Other frequent findings in affected individuals include defects in the cardiac conduction system, cerebral ataxia and high concentration of proteins in the cerebro-spinal fluid (>100 mg/dl).

Patients with SKS exhibit bilateral ptosis as one of the first signs of the disease followed by chronic progressive external ophthalmoplegy a few years later. In our case the lateroversions as well as the supra- and infra-version were involved, indicating a severe form of the disease. The defects in the cardiac conduction system are common in the syndrome as they appear in 57% of patients. In our case, the only electrocardiographical evidence was a slight incomplete blockage. These data indicate a phenotype heterogeneity in the SKS which can pose challenges and difficulties in the diagnostic of this syndrome in some patients. In this context it is interesting to note that the «common deletion» of the SKS in the mtDNA is closely related to cardiac conduction alterations (8.15). The instant case proves and confirms said correlation. Cardiovascular follow-up is indicated in all SKS patients in order to identify and treat at an early stage the severe alterations and complications which appear later on.

In SKS as well as in other disorders related to mtDNA heteroplasmy can be found, a mixture of mutant and wild mtDNA in the same cell or tissue. The relationship of mutant DNA with wild DNA is of great importance to determine the severity of the phenotype in a mitochondrial disorder. The absence of cerebral ataxia and neurosensory deafness in our patient could be due to this. On the other hand, skeletal muscle biopsy is one of the usual methods for a histopathological diagnosis of SKS, with the red-torn fibers being the characteristic finding in the affected muscle.

Table I. Sequence of primers utilized in the mtDNA deletion study. Numbers in brackets indicate nucleotide position

<table>
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<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>MT-1 (8468)</td>
<td>5´- CTT TGA AGT AGG GCC GTT TAC - 3´</td>
</tr>
<tr>
<td>MT-4 (13707)</td>
<td>5´- CTG CGA ATA GGC TTC CGG CTG CC - 3´</td>
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Fig. 4: PCR amplified mtDNA products. Line 2: DNA control showing a high molecular weight range corresponding to normal ~5 kb mtDNA; line 3: patient DNA showing a 550 pb range indicating common deletion and a less intense ~5 kb range corresponding to the wild mtDNA, showing mitochondrial heteroplasmy.
However, the development of non-invasive diagnostic procedures such as the demonstration of SKS «common deletion» by means of PCR can avoid the need of taking biopsies, thus facilitating the approach of patients with suspected SKS. PCR is a useful alternative for cases in which the patient rejects a muscle biopsy as a diagnostic method.

The PCR methods for detecting «common deletion» of SKS are based on the fact that if the is absent the 5 kb fragment will not be detected due to its excessively large size for amplification. On the contrary, if the deletion is present, the small mtDNA remainder fragment will be easily detected and amplified (10,13). A clear drawback of this approach is the impossibility of detecting heteroplasmia. In the method described herein it was possible to amplify the small fragment indicating the SKS «common deletion» as well as the ~5 kb fragment of wild mtDNA, thus demonstrating the presence of heteroplasmic mitochondria. Similarly, this method could have detected homoplasmia for mutant or normal mtDNA in the tissue under study.

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5. Kearns TP, Sayre GP. Retinitis pigmentosa, external ophthalmoplegia and complete heart block: unusual syndrome with histologic study in one of two cases. AMA Arch Ophthalmol 1958; 60: 280-289.