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Do Mitochondrial Diseases Have a Role in the Pathogenesis of Obstructive Sleep Apnea?

Mitokondriyal Hastalıkların Obstrüktif Uyku Apnesinin Patogenezinde Rolü Var mı?

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ABSTRACT

Objective: This study was designed to investigate and compare histopathological changes in pharyngeal musculature in simple snorers and in patients with obstructive sleep apnea hypopnea syndrome (OSAHS).
Material and Methods: Fifteen patients with OSAHS and seven simple snorers were included in the study. A cylindrical shape of muscle tissue (about 1 cm long x 0.5 cm wide) was obtained from the palatoglossus muscle during surgery. Samples stained with haemotoxylin/eosin and nicotinamide adenine dinucleotide (NADH) tetrazolium reductase. Proportion of type I and type II fibers, group atrophy, and type grouping were noted. Mitochondrial disease (MD) was suspected in two of the OSAHS patients and modified Gomori trichrome, succinate dehydrogenase (SDH), and double staining with cytochrome c-oxidase (COX) and SDH were performed to all cases.
Results: Type grouping and group atrophy were not detected in both of the groups. Type II fibers were found to be predominant in seven OSAHS patients and one simple snorer and there was no significant difference between the two groups (p=0.19). COX deficiency, indicating mitochondrial disease was detected in two OSAHS patients. COX-deficiency was not significantly different (p>0.45) between the simple snorers (0/7) and OSAHS patients (2/15).
Conclusions: Although rare, detection of mitochondrial muscle disease in some patients with OSAHS; let us concentrate on a new research subject that may have a role in the pathogenesis of upper airway collapse in patients with OSAHS.

Keywords
Sleep apnea, obstructive, mitochondrial myopathies

ÖZET

Amaç: Bu çalışmada obstrüktif uyku apne hipopne sendromlu (OSAHS) ve basit horlaması olan hastaların faringeal kaslarındaki histopatolojik değişiklikleri araştırmak ve karşılaştırmak amaçlanmıştır.
Yöntem ve Gereçler: Çalışmamıza 15 OSAHS ve yedi basit horlama hastası dahil edilmiştir. Cerrahi sırasında hastaların palatoglossus kasından yaklaşık 1 cm boyunda ve 0,5 cm eninde silindirik şekilde doku örnekleme için alınmıştır. Örnekler hemotoksilen/eosin ve nikotinamide adenin dinucleotid (NADH) tetrazolyum reduktaz ile boyanmıştır. Örnekler, tip I ve tip II lifler arasındaki oran, grup atrofisi ve tip gruplaması açısından incelenmiştir. OSAHS'ı olan iki hastada mitokondriyal hastalıktan şüphelenilmiş ve bunun üzerine tüm spesimenlere modifiye Gomori trikrom, süksinat dehidrogenaz (SDH) ve sitokrom c-oksidaz (COX) ve SDH ile çift boyama uygulanmıştır.
Bulgular: Her iki grupta da tip gruplaması ve grup atrofisi tespit edilememiştir. Yedi OSAHSlı ve bir basit horlaması olan hastada tip II liflerin baskın olduğu görülmüştür ancak lif tiplerinin dağılımı iki grup arasında anlamlı fark yaratmamıştır (p=0.19). Mitokondriyal hastalığın bulgusu olan COX eksikliği iki OSAHS hastasında tespit edilmiştir. COX eksikliğinin dağılımı, basit horlaması olan hastalar (0/7) ve OSAHS hastaları (2/15) arasında istatistiksel olarak anlamlı bulunmamıştır (p>0.45).
Sonuç: Nadir olsa da OSAHS hastalarının bazılarında mitokondriyal kas hastalığı tespit edilmesi, bizi OSAHS hastalarında görülen üst havayolu obstrüksiyonunun patogenezinde rol alabileceğini düşündüğümüz yeni bir araştırma konusuna yoğunlaşmaya yöneltmiştir.

Anahtar Sözcükler
Obstruktif uyku apnesi, mitokondriyal myopatiler

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INTRODUCTION

Obstructive sleep apnea hypopnea syndrome (OSAHS) is characterized by recurrent episodes of partial or complete obstruction of the upper airway. This disorder commonly causes intermittent hypoxemia and sleep fragmentation. The prevalence of OSAHS in adults is 4-9%, and it has a strong association with significant increases in morbidity and mortality, as well as with decreased quality of life.[1] However, the pathophysiological mechanisms for the collapse of the upper airway during sleep in OSAHS patients are not well defined yet.

Most studies suggest that adults with OSAHS have a narrower upper airway than non-OSAHS subjects.[2,3] It is likely that larger than normal soft tissue structures, such as the soft palate, tongue, parapharyngeal fat pads, tonsils, and lateral pharyngeal walls interact to narrow the upper airway lumen. Although the preceding evidence has shown that structural factors play an important role, it is impossible to explain the entire pathogenesis of upper airway collapse by anatomical factors alone. It is agreed that the ability of upper airway muscles to develop a dilating force to counterbalance the effects of collapsing forces plays a critical role in the pathophysiology. There is an accumulation of evidence indicating the role of neuromuscular factors in the development of upper airway collapse.[4,5]

Histological changes associated with OSAHS have been investigated extensively in recent years and various morphological abnormalities indicating neuromuscular disorders were demonstrated in pharyngeal muscles. Edstrom et al.[6] studied biopsies of palatopharyngeal muscle samples from OSAHS patients and normal controls. Only biopsies from OSAHS patients showed type grouping, an indicator of a neurogenic lesion. These authors suggest that the neurogenic lesion might be a primary phenomenon or might be secondary to trauma; specifically, stretching of the pharyngeal tissues during snoring and repetitive apneas. Friberg et al.[7] performed further histological and electron microscopic investigations of the pharyngeal muscles in OSAHS patients. Similarly, their results showed lesions consistent with polyneuropathy. Additionally they also demonstrated afferent nerve lesions of the palatal mucosa immunohistochemically in OSAHS patients.[8] Woodson et al.[9] showed focal degeneration of myelinated nerve fibers in patients with severe sleep apnea. Smirne et al.[10] found an abnormal distribution of fiber types in the pharyngeal constrictor muscles of habitual snorers.

The aim of the present study was to investigate and compare histopathological changes in the pharyngeal musculature in simple snorers and OSAHS patients. Surprisingly, signs indicating mitochondrial disease (MD) were observed in some of the OSAHS patients. The presentation of a relatively rare disease (MD) in our 15-patient OSAHS group may lead to the identification of new contributing factors in the pathophysiology of obstructive sleep apnea.

MATERIAL AND METHODS

Subjects

Among the candidates scheduled for uvulopalatopharyngoplasty (UPPP) or uvulopalatal flap (UPF) surgery at the Hacettepe University Faculty of Medicine, Department of Otorhinolaryngology Head and Neck Surgery between January and December 2004, 22 patients agreed to participate were included in the study. The definitive diagnosis of OSAHS or simple snoring was based on overnight polysomnography. OSAHS was considered to be present if AHI was ≥ 5. Patients were grouped as simple snorers and OSAHS patients. In all, 7 patients (31.8%) with an AHI < 5 were diagnosed as simple snorers. Of the remaining 15 patients (68.2%), 6 had an AHI between 5 and 14.9 (mild OSAHS), 6 had an AHI between 15 and 29.9 (moderate OSAHS), and 3 had an AHI ≥ 30 (severe OSAHS). The study protocol was approved by the Hacettepe University Ethics Committee and all patients provided written informed consent to participate in the study.

Muscle sampling and analysis

A cylindrical shape of muscle tissue (about 1 cm long x 0.5 cm wide) was obtained from the palatoglossus muscle during UPPP or UPF surgery. Muscle samples were rapidly frozen by submersion in isopentane, cooled in liquid nitrogen (-160°C) and then successive transverse sections (10 μm) were cut with a cryostat. All muscle specimens were stained with haemotoxylin and eosin, and nicotinamide adenine dinucleotide (NADH) tetrazolium reductase. After the staining procedure all specimens were examined under light microscopy. Variability in fiber size, the presence of internal nuclei, proportion of type I and type II fibers, group atrophy, and type grouping were noted. As increased enzyme activity was observed in the NADH stained sections of two

of the OSAHS patients, in order to investigate suspected mitochondrial disease modified Gomori trichrome, succinate dehydrogenase (SDH), and double staining with cytochrome c-oxidase (COX) and SDH were performed for all cases. Medical records of the two patients who diagnosed with MD were reviewed in respect.

The data of the simple snorers and OSAHS patients were compared with the chi-square test.

RESULTS

The study included 22 patients (17 males, 5 females). Demographic data, AHI, and histopathological data of all 22 patients are summarized in Table 1. Mean AHI was 3.2 (range: 1.6-4.8) and 20.3 (range: 9-40.3) in simple snorers and OSAHS patients, respectively. Mean age of simple snorers (6 males, 1 female) was 39.9 ± 11.4 years and the mean age of OSAHS patients (11 males, 4 females) was 47.3 ± 8 years. There was no significant differences in the distribution of sex or age (p=0.51 and p=0.09 respectively) between the simple snorers and OSAHS patients. All of the OSAHS patients and two simple snorers underwent UPPP surgery, while the remaining 5 simple snorers underwent UPF surgery.

The fiber sizes ranged from 20 µm to 80 µm in the specimens. None of the patients had an increase in the number of internal nuclei. Type II fiber predominance was observed in seven OSAHS patients (46%) and in one simple snorer (14%). In the remaining patients type I and type II muscle fibers were distributed equally. Type II fiber predominance in the simple snorers and OSAHS patients was not significantly different (p=0.19). Group atrophy and type grouping were not detected in both simple snorers and OSAHS patients.

COX-deficient fibers were observed in two OSAHS patients (Figure 1). Each patient showed five fibers per small power view (10x). In one patient ragged red fibers were also detected (Figure 2). The ages of the patients were 50 and 52. The medical records of these two patients revealed no specific information about MD. One of these patients had moderate OSAHS, while the other had severe OSAHS. None of the simple snorers had COX-negative fibers. COX-deficiency was not significantly different (p>0.45) between the simple snorers (0/7) and OSAHS patients (2/15).

DISCUSSION

Mitochondrial diseases are a heterogeneous group of genetic disorders with widely varying clinical features, which are the result of defects in mitochondrial function. Deficiency in mitochondrial energy production can originate in many different segments of the biochemical energy production mechanism of the

Tablo 1. The demographic data, AHI (apnea hypopnea index), and histopathological data of all 22 patients.

	Sex	Age	AHI	Fiber size (µm)	Internal nuclei	Fiber type	Type grouping	Group atrophy	Cox deficiency
1	M	53	12	20-80	-	II>I	-	-	-
2	F	50	40,3	20-80	-	II>I	-	-	-
3	M	47	9	20-60	-	II=I	-	-	-
4	F	47	22.1	28-80	-	II>I	-	-	-
5	M	39	27.4	20-60	-	II=I	-	-	-
6	M	38	1.6	28-96	-	II=I	-	-	-
7	M	22	1.6	40-80	-	II=I	-	-	-
8	M	46	14	20-72	-	II>I	-	-	-
9	F	48	4.8	28-52	-	II=I	-	-	-
10	M	35	4	20-60	-	II=I	-	-	-
11	M	28	24.3	20-60	-	II>I	-	-	-
12	M	38	19.3	20-48	-	II=I	-	-	-
13	M	32	4	24-64	-	II>I	-	-	-
14	M	50	17	24-60	-	II>I	-	-	+
15	M	54	3.2	24-68	-	II=I	-	-	-
16	F	56	22,6	20-40	-	II=I	-	-	-
17	M	48	9	20-40	-	II=I	-	-	-
18	M	50	2.9	20-60	-	II=I	-	-	-
19	F	55	30.8	20-40	-	II=I	-	-	-
20	M	52	39	20-52	-	II>I	-	-	+
21	M	42	9	20-60	-	II=I	-	-	-
22	M	59	9.2	20-52	-	II=I	-	-	-

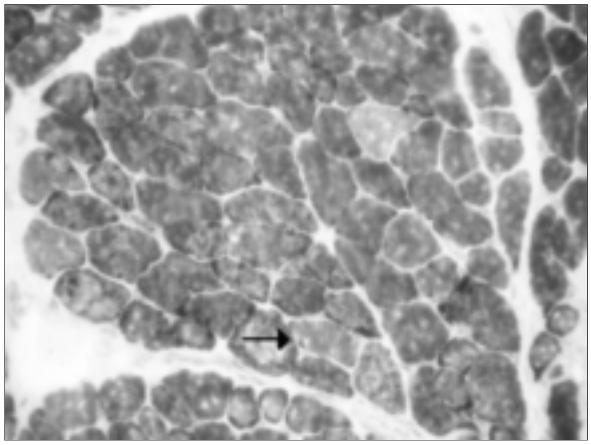

Figure 1. Double staining with COX and SDH stains. COX negative fibers are seen as blue . Several COX-negative fibers are present. (arrow indicates a COX negative fiber).

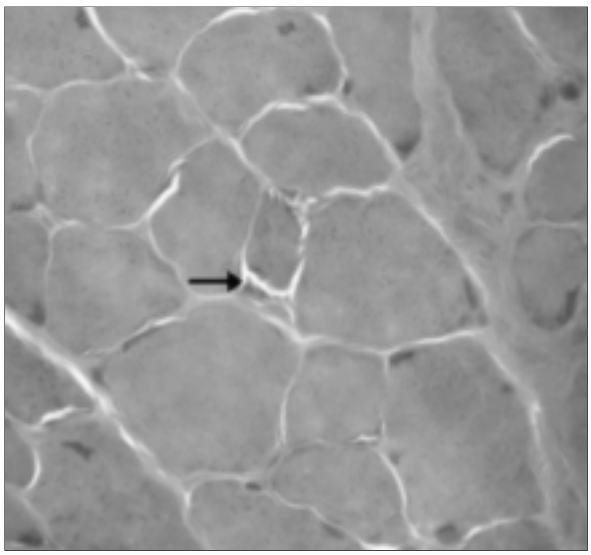

Figure 2. Muscle biopsy from palatoglossus muscle shows fiber size variability, round fibers, and a "ragged red" fiber. (arrow indicates a ragged red fiber).

mitochondrion. These disturbances are due to different genetic defects in both nuclear and mitochondrial DNA.[11-13] Muscle, because of its high-energy requirements and dependence on oxidative metabolism, is one of the tissues most commonly affected by mitochondrial dysfunction, and myopathy is present in many of the known MD. It is becoming clear that peripheral neuropathy is also an important manifestation of mitochondrial dysfunction.[11]

MD may present at any age. The clinical presentation of MD has a wide spectrum, ranging from severe neuromuscular disorders to non-specific mild muscle weakness.[14] Exercise intolerance is a well-recognized clinical feature of MD and exercise capacity decreases in proportion to the increasing mutation load in muscle.[13] Exercise intolerance or weakness of the limbs is the presenting complaint in 23% of patients and times may be the only manifestation of mitochondrial disease.[15]

The clinical and genetic heterogeneity exhibited by MD poses a diagnostic challenge. If the history, physical examination, and supportive laboratory tests suggest mitochondrial disease, the next step is to determine whether the patient has a defined syndrome or disease associated with mitochondrial disorder. If the patient does not have a classical syndrome, muscle biopsy should be performed. The analysis of the specimen should include an investigation of ragged red fibers or COX-negative fibers.[11] The SDH reaction clearly shows the sub-sarcolemmal accumulation of the mitochondria in MD. The presence of low levels of COX-deficient fibers must be interpreted with caution. Clonal expansion of mitochondrial DNA deletions in single fibers is well recognized in aging muscle and these deletions will manifest as focal COX defects.[16] The presence of COX-negative fibers in the elderly (> 70 years old) emphasizes the importance of obtaining additional clinical, biochemical and genetic data.[17] The prevalence of MD leading to disorders in patients of working age (16-60 years) was reported to be between 1/15,000 and 1/17,500 in various studies.[18,19] In the present study we diagnosed MD in two OSAHS patients; they were 52 and 50 years old, who are not old enough to have COX-deficient fibers characteristic of old age. The observed positive histological signs of MD were not significantly different between the simple snorers (0/7) and OSAHS patients (2/15) in our study group, but the MD frequency (2/15, 13.3%) was relatively high. We think that the diagnosis of such a rare disease in our very limited patient population was beyond chance; however stressing MD as a factor in the pathophysiology of OSAHS seemed to be impossible based on our limited data. Furthermore, because we did not design the study for investigating MD in our patient group, we did not perform detailed examinations of the patients for systemic signs and symptoms of MD. Additionally, neither biochemical assessments of mitochondrial function nor genetic analysis could be performed to confirm the pathological diagnoses. Despite all of these limitations, we suggest that local muscle weakness due to mitochondrial dysfunction might have played a role in the pathogenesis of upper airway collapse.

There are numerous well-known mitochondrial and nuclear DNA mutations that cause MD. Different genetic and non-genetic factors, such as the individual energy demand of each tissue, age, type of gene involved, and DNA mutation result in different clinical phenotypes and different clinical presentations. Progressive external ophthalmoplegia is the most important example of mitochondrial myopathy and affects the extraocular muscles locally. Similarly, in obstructive sleep apnea pharyngeal muscles may be involved locally with a subclinical form of mitochondrial myopathy and decreased exercise intolerance of pharyngeal muscles may interact with obstruction of the upper airway.

Peripheral neuropathy is reported with increasing frequency in MD.[15-20] Most of the previous histopathological studies concerning OSAHS reported neurogenic lesions, such as peripheral neuropathies. In addition to vibratory trauma, MD may contribute to the formation of neuropathy in pharyngeal muscles in OSAHS patients. To validate these hypotheses, the necessity of further investigations, including histopathological, histochemical, biochemical assessment of mitochondrial function in pharyngeal musculature, and molecular genetic analysis of high number of OSAHS patients, is obvious.

In the present study we also investigated the morphological signs of localized myogenic or neurogenic lesions. Smirne et al.[10] found that habitual snorers had an abnormal distribution of fiber types compared to non-snorers: the number of type I and type IIB muscle fibers were reduced, whereas type IIA fibers increased in number. Smirne et al.[10] suggested that a reduction in the number of motor neurons could induce adaptive transformation and hypertrophy of type II fibers. In our study we found type II fibers were predominant in 7 of 15 OSAHS patients and in 1 of 7 simple snorers: the difference between the groups was not significant but the abnormal distribution of muscle fibers supported the possibility of a neurogenic lesion in the pharyngeal musculature in some of the OSAHS patients and simple snorers.

Degeneration of motor nerves leads to a lack of electro-mechanical activity in muscle fibers. Re-innervation of denervated muscle fibers can originate due to a destroyed axon by regeneration or by collateral sprouting from a nearby intact axon. The re-innervation process may cause type grouping. If that axon again becomes denervated, the muscle fibers of that motor unit will atrophy. Such a motor unit will display not only type grouping, but also grouped atrophy.[5] In contrast to the literature, the well-known indicators of a neurogenic lesion, type grouping and grouped atrophy were not observed in either the OSAHS patients or simple snorers. Detection of internal nuclei in at least 10% of the fibers is suggestive of myopathy.[10] Internal nuclei could not be detected in any of our patients.

CONCLUSION

Positive histological signs of MD were not significantly different between the simple snorers and OSAHS patients in our study group but diagnosing MD in patients with OSAHS seems to be important. Further investigations, including histochemical, biochemical assessments of mitochondrial function, and genetic analysis in OSAHS patients may help enlightening the subject.

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