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Discoveries

NEWS & VIEWS

Can Mitochondrial Dysfunction Be a Predictive Factor for Oxidative Stress in Patients with Obstructive Sleep Apnea?

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Abstract

Mitochondrial dysfunction reflects a lifelong cumulative burden of cellular damage, and a decrease in mitochondrial DNA (mtDNA) copy number is associated with oxidative stress and chronic inflammation. The goal of this study was to assess whether mitochondrial dysfunction and a decrease in mtDNA copy number are common features of patients with obstructive sleep apnea syndrome (OSA). We compared mtDNA copy number between 20 healthy volunteers and 20 patients with OSA and investigated whether a significant attenuation of mtDNA copy number was observed in genomic DNA isolated from whole blood of OSA patients. Our observations lead to the hypothesis that mtDNA copy number is lower in whole blood DNA of OSA subjects and might be related to OSA severity, reflecting excessive oxidative stress in patients with OSA. *Antioxid. Redox Signal*. 21, 1285–1288.

Mitochondrial Function and Obstructive Sleep Apnea Syndrome

OBSTRUCTIVE SLEEP APNEA SYNDROME (OSA) is characterized by repeated events of partial or complete airway obstruction that reduce or interrupt airflow, disrupt normal ventilation, and cause sleep fragmentation. Increasing evidence suggests that OSA is pathophysiologically linked to intermittent hypoxia or hypoxemia and is an important risk factor for cardiovascular diseases. It is well known that OSA might lead to hypertension, stroke, ischemic heart disease, arrhythmia, congestive heart failure, and atherosclerosis in the absence of proper diagnosis and treatment (2, 5, 6). Therefore, effective diagnosis, treatment, and prevention of OSA are very important. Additionally, several studies have focused on predicting the complications of OSA, including cardiovascular diseases (2, 5).

Oxidative stress is believed to be a potent factor in cellular damage because oxidative stimuli affect protein conformation, catalytic activity, protein–protein interactions, and protein–DNA interactions. OSA is associated with intermittent hypoxia during sleep and it is possible that excessive oxidative stress in OSA patients who are not treated efficiently might cause cardiovascular complications (3). We hypothesized that biological markers of oxidative stress might predict cardiovascular risk and be beneficial in preventing complications in OSA patients.

Mitochondria play essential roles in cell proliferation, differentiation, and apoptosis. Functional changes in mitochondria

Innovation

The fatal cardiovascular complications in obstructive sleep apnea syndrome patients can be mediated by intermittent hypoxia-induced sympathetic activation and oxidative stress during sleep. Immoderate mitochondrial dysfunction which has been found in age-related disorders and chronic inflammation might be correlated with excessive free radical stimulation and oxidative stress. In the present study, we observed that mitochondrial dysfunction was more characteristic in whole blood from OSA patients and was closely correlated with degree of oxygen desaturation or severity of OSA. We also estimated that mtDNA copy number might reflect the degree of oxidative stress and be predictive of cardiovascular risks in patients with OSA.

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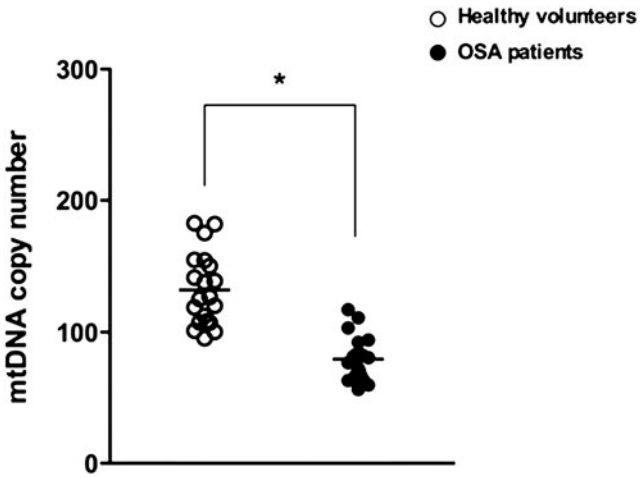

FIG. 1. Mitochondrial DNA (mtDNA) copy number in healthy volunteers ($n=20$) and obstructive sleep apnea syndrome (OSA) patients ($n=20$). Real-time polymerase chain reaction showed that mtDNA copy number in whole blood DNA was significantly lower in OSA patients than in healthy volunteers. Graphs show mean values (*$p<0.05$ when compared with healthy volunteers).

might be associated with cellular damage, stress-related conditions or disorders, and loss of energy (1). Systemic inflammation and metabolic disturbance can induce excessive mitochondrial dysfunction, and these conditions might be correlated with increased oxidative stress in OSA patients.

Mitochondrial Dysfunction in the Peripheral Blood of OSA Patients

The purpose of this study was to compare mitochondrial DNA (mtDNA) copy number in whole blood genomic DNA of OSA patients and healthy volunteers. We also estimated the degree of oxidative stress by evaluating mitochondrial dysfunction in OSA patients.

The mean mtDNA copy number in whole blood DNA was significantly lower in the 20 OSA patients compared with the 20 healthy volunteers (133.12 ± 33.0 for OSA *vs.* 83.92 ± 17.3 for controls, $p<0.05$) (Fig. 1).

The mean mtDNA copy number in whole blood DNA samples of healthy volunteers younger than 20 years was 158.72 ± 26.1, and this value gradually decreased with increasing age. The mean mtDNA copy number in whole blood DNA of healthy volunteers older than 60 years was 118.65 ± 11.5. In contrast, the mean mtDNA copy number in whole blood DNA samples of OSA patients aged 20–40 years was 95.0 ± 19.8, which was significantly lower than the mtDNA copy number measured in samples from healthy volunteers of the same age. The mean mtDNA value was 69.32 ± 9.5 in OSA patients aged 40–60 years and 75.2 ± 5.6 in OSA patients older than 60 years. In the 40–60 and older than 60 age groups, mtDNA values of OSA patients were lower than those of healthy subjects (Fig. 2A). The mtDNA copy number decreased more acutely with age in OSA patients compared with healthy volunteers.

The mean mtDNA copy number in whole blood DNA of healthy volunteers with BMI under 22.9 kg/cm^2 was 140.78 ± 24.1, and the copy number gradually decreased as BMI increased. The mean mtDNA copy number in whole blood DNA of healthy volunteers with BMI >25.0 kg/cm^2 was 115.78 ± 14.6. The mean mtDNA copy number in whole blood DNA of OSA patients with BMI <22.9 kg/cm^2 was 80.2 ± 32.0 and that of OSA patients with BMI from 23.0 to 24.9 kg/cm^2 was 96.52 ± 10.9. The lowest mtDNA copy number was observed in OSA patients with BMI >25.0 kg/cm^2 (72.57 ± 9.8). The mean mtDNA copy numbers were significantly lower than the values of healthy subjects in the same BMI group (Fig. 2B). The mtDNA copy number also decreased more acutely with increasing BMI in OSA patients than in healthy volunteers.

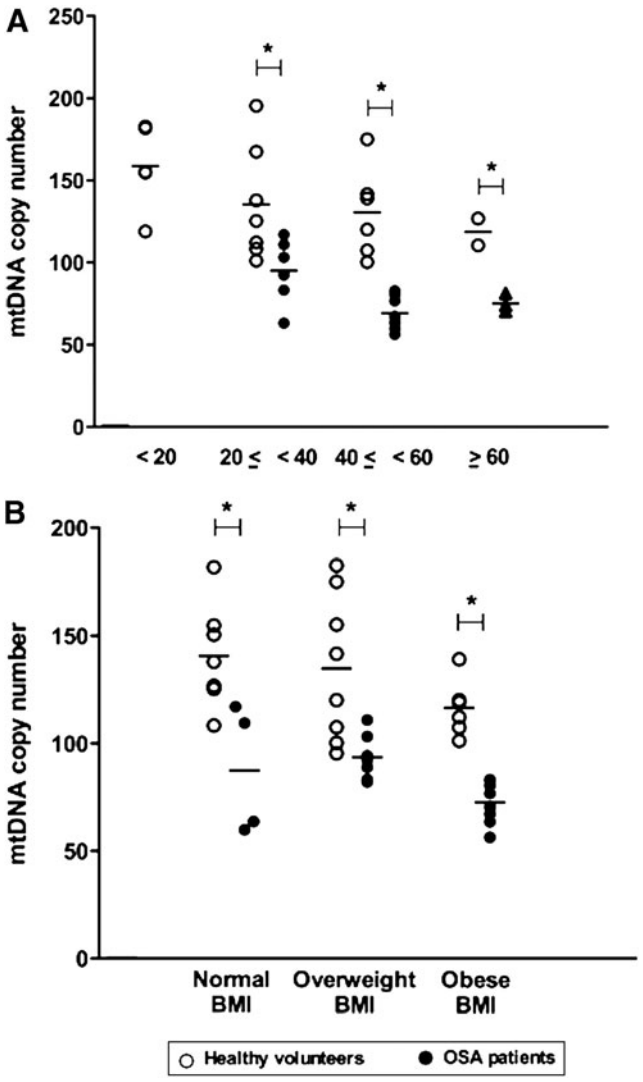

FIG. 2. The change of mean mtDNA copy number as age- and BMI-dependent manners. The mtDNA copy number in the whole blood DNA samples of healthy volunteers ($n=20$) and OSA patients ($n=20$) compared by age group **(A)**. The mtDNA copy numbers in whole blood DNA of healthy volunteers ($n=20$) and OSA patients ($n=20$) compared by BMI **(B)**. Graphs show mean values. *White dot*: mtDNA copy number and telomere length of healthy volunteers; *black dot*: mtDNA copy number and telomere length of OSA patients (*$p<0.05$ comparing healthy and OSA patients).

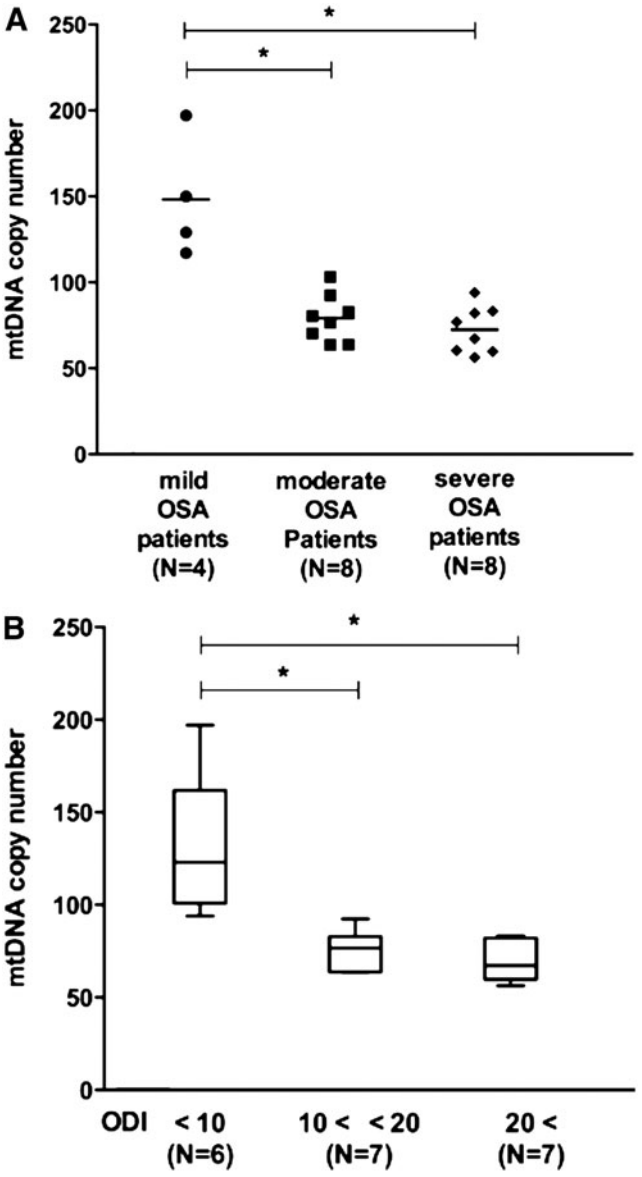

FIG. 3. The change of mean mtDNA copy number as OSA severity and oxygen desaturation. Comparison of mtDNA copy number in whole blood DNA of OSA patients ($n = 20$) depends on OSA severity **(A)**. Comparison of mtDNA copy number in whole blood DNA samples of OSA subjects ($n = 20$) and dependence on oxygen desaturation **(B)**. Graphs show mean value and $*p < 0.05$ compared to patients with mild OSA (*circle*: mild OSA patients, *rectangle*: moderate OSA patients. *diamond*: severe OSA patients).

Low mtDNA Copy Number in OSA Patients Correlated with OSA Severity and Oxygen Desaturation

The mean mtDNA copy number for the four patients with mild OSA was 87.47 ± 22.9, which was significantly higher than that in the eight patients with moderate OSA (79.78 ± 18.6, $p < 0.05$). The lowest mtDNA copy number (74.9 ± 12.4) was observed in the eight patients with severe OSA. The mean mtDNA copy number of patients with severe OSA was significantly lower than that in patients with mild OSA (Fig. 3A).

We classified OSA patients according to the oxygen desaturation index (ODI) and compared the mean values of mtDNA copy number. Six OSA patients had ODI lower than 10; seven had ODI of 10 to 20; and seven had ODI greater than 20. The mean mtDNA copy number was 131.71 ± 37.5 in OSA patients with ODI < 10, 75.71 ± 10.6 in patients with ODI 10–20, and 69.42 ± 11.2 in patients with ODI > 20; the last two values were significantly lower than the value of OSA patients with ODI < 10 (Fig. 3B). These results indicate that decreased mtDNA copy number was related to OSA severity and was observed in OSA patients with a lower ODI.

Could mtDNA Copy Number Be a Predictive Marker for Oxidative Stress of OSA Patients?

Unbalanced oxidative stress can have important clinical implications in OSA patients and might cause fatal cardiovascular complications (6). Increased reactive oxygen species and decreased antioxidant capacity in OSA patients might be important in the pathogenesis of cardiovascular complications, providing motivation for appropriate treatment. The ability to predict the amount or burden of oxidative stress might therefore be important for the prevention of cardiovascular complications in OSA. Our data showed that the degree of oxygen desaturation was clinically related to the amount of oxidative stress, and we assume that precise measurement of biomarkers for oxidative stress, such as mtDNA copy number, might show potential utility to predict cardiovascular complications in OSA patients.

Our findings provide evidence that intermittent hypoxia in patients with OSA enhanced systemic oxidative stress, resulting in mitochondrial dysfunction in the genomic DNA of whole blood cells. In addition, a significant correlation was found between OSA severity and decreased mtDNA copy number, suggesting that OSA patients with higher apnea –hypopnea index and higher severity are exposed to greater systemic blood oxidative stress and, if left untreated, cardiovascular complications might be more likely in these patients.

The pathogenesis of cardiovascular diseases might be closely related to sympathetic activation, vascular endothelial dysfunction, chronic inflammation, and hypercoagulability, and these cardiovascular pathologies can be caused by oxidative stress (7). We presume that OSA patients are exposed to repetitive apneic episodes and intermittent hypoxia that might cause systemic oxidative stress. Prospective studies show that untreated OSA is associated with increased mortality and that controlling for airway collapse is needed to avoid fatal complications of OSA. The association between OSA and increased mortality is partly explained by a higher prevalence of cardiovascular disease (4). Therefore, clinicians should be aware of the association between OSA and increased mortality and should attempt to predict cardiovascular disease in OSA patients. We propose that reduced mtDNA copy number in whole blood from OSA patients might be a representative biomarker for adverse outcomes from cardiovascular complications. Moreover, mtDNA copy number in whole blood of OSA subjects provides predictive insights into the significance of oxidative stress and cellular aging if OSA is not treated appropriately.

Conclusions

The main and novel finding of this study is that mtDNA copy number was significantly reduced in whole blood DNA of patients with OSA. These changes appeared to be

dependent on the degree of airflow cessation due to apneic events and the degree of oxygen desaturation. Our findings suggest that mtDNA copy number is representative of the oxidative stress of OSA patients and could be a reliable marker for predicting cardiovascular risk in patients with OSA.

Notes

Subjects and methods

This study enrolled 40 people who were referred to the Sleep Disorder Center in the Department of Otolaryngology and Head & Neck Surgery at the Chung-Ang University College of Medicine (Seoul, Korea) between July 2011 and June 2013. The sleep reports of 20 adults diagnosed with OSA and medical records of 20 healthy volunteers were reviewed retrospectively.

mtDNA was extracted from 2 ml of whole blood using commercial kits (Qiagen, Inc., Valencia, CA). Relative mtDNA copy number was measured using real-time polymerase chain reaction (PCR) with a Light Cycler-Fast Start DNA Master SYBR Green I kit (Roche Molecular Biochemicals, Pleasanton, CA). The number of PCR cycles required for 20 ng DNA was defined as the threshold cycle number (Ct), and mtDNA copy number was calculated using the following equation: relative copy number $= 2\Delta Ct$ ($\Delta Ct = Ct\beta\text{-globin} - CtND1$).

Statistical analysis

Data are presented as mean ± standard deviation for normal distribution, median with interquartile range (IQR, 23th–75th percentile) for nonnormal distribution, or number (%) for categorical variables. The mtDNA copy numbers were logarithmically transformed before statistical analyses to approximate a normal distribution. Pearson correlation coefficients were calculated to evaluate the relationships between mtDNA copy number and continuous variables. Significance was defined at the 0.05 level of confidence. We performed a stepwise multiple linear regression analysis to exclude the influences of potential confounding variables. All analyses were performed with SPSS (version 18.0; SPSS, Inc., Chicago, IL) for Windows software. A p-value < 0.05 was considered statistically significant.

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Abbreviations Used

mtDNA = mitochondrial DNA
ODI = oxygen desaturation index
OSA = obstructive sleep apnea syndrome
PCR = polymerase chain reaction