

Effect of Sleep Quality on Mitochondrial DNA Copy Number in Eveningness Chronotypes

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ABSTRACT

Objective: To determine the effect of sleep quality on mitochondrial DNA copy number in eveningness chronotype.

Study Design: Cross-sectional study.

Place and Duration of the Study: Department of Physiology, Islamic International Medical College, in collaboration with Genetic Resource Centre, from August 2022 to May 2023.

Methodology: A total of 80 participants with eveningness chronotype based on the Morningness-Eveningness Questionnaire were recruited. The participants' sleep quality was assessed using Pittsburg Sleep Quality Index (PSQI). Accordingly, they were categorised into Group A (good sleep quality) and Group B (poor sleep quality) with 40 participants in each group. After extracting DNA using the Chelex method, mitochondrial DNA copy number of all participants was measured using the quantitative polymerase chain reaction. The mean values in both groups were analysed by Independent-samples t-test.

Results: Analysis of mitochondrial DNA copy number in both groups showed normal distribution after being transformed into logarithmic values. The mean value of Group A (2.65 ± 0.26) exhibited a significant increase in mitochondrial DNA copy number (95% CI: 0.18, 0.44; $p < 0.001$) as compared to that in Group B (2.34 ± 0.29).

Conclusion: People with good sleep quality have a higher mtDNA-CN than those with poor sleep quality. Good sleep quality may counteract negative effects of increased oxidative stress brought on by eveningness behaviour, thus leading to better mitochondrial function and increased mitochondrial biogenesis that was indicated by higher mtDNA-CN in individuals who experience good sleep quality.

Key Words: Mitochondria, Mitochondrial DNA copy number, Sleep quality, Chronotype.

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INTRODUCTION

Mitochondria are double membranous organelles that are involved in a wide range of biological processes such as ATP synthesis, metabolism, apoptosis, and inflammation. Mitochondria have their own genomes, mitochondrial DNA (mtDNA), that can have tens to thousands of copies per cell. This quantity variation is known as mitochondrial DNA copy number (mtDNA-CN), which varies greatly between cell types and individuals.¹ The mtDNA-CN has been postulated as a viable biomarker of ageing and an effective focus of stress including physiological and environmental stress.^{2,3} Numerous studies had shown that mitochondrial dysfunction led to abnormal cellular energy production, nuclear gene expression, and excessive reactive oxygen species (ROS) production, which all contributed to chronic diseases like cardiovascular diseases and Type 2 Diabetes mellitus (T2DM).²

Sleep is a restorative process that allows the brain and body to recover from the activities that occur during waking hours. This repair not only allows for energy and mental concentration regeneration but is also proposed to offer an opportunity for cellular restoration.⁴ Sleep quality is described as an individual's degree of satisfaction while sleeping and includes four components: sleep efficiency, sleep latency, sleep duration, and waking after sleep commences.^{5,6} According to the National Sleep Foundation recommendations, younger persons should sleep for seven to nine hours, while older ones should sleep for seven to nine hours.⁷ Sleep continuity, which refers to the uninterrupted and effortless transition through the various phases of sleep without frequent awakenings or disturbances, is another significant aspect of sleep quality. It assesses how well a person sleeps continuously and uninterrupted during the night.⁵ Sleep continuity is an important aspect of sleep quality as it directly affects the restorative and rejuvenating functions of sleep.⁸ Numerous studies showed that sleep quality has been declining in recent years, particularly among young individuals.⁷ Sleep quality is affected by several factors including environmental factors (noise, light levels, room temperature), lifestyle factors (sleep schedule, physical activity, eating and drinking habits and use of electronic devices) and psychological factors (stress, anxiety, mental health conditions).^{6,9}

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Chronotype is the term used to describe a person's innate propensity to sleep and wake up at particular times of the day or night, which is impacted by their biological clock as well as external variables including heredity and environment. Work performance, mood, and general health are just a few areas of daily living that chronotype can affect. Chronotype can be classified into three categories; morningness chronotype (MC) who prefers early bed and wake time, eveningness chronotype (EC) who prefers a later bedtime and later wake time, and intermediate who lies in between the two.¹⁰

Numerous health-related variables and chronotypes had been linked in studies. Compared to morningness chronotype, eveningness chronotype tends to have more health issues, such as psychological illnesses, cardiovascular diseases, and higher mortality. Additionally, it had been noted that eveningness chronotype is more likely to acquire metabolic illnesses such as Type 2 Diabetes and the metabolic syndrome.¹¹ However, data on the effects of sleep quality on mtDNA-CN in eveningness chronotype is sparse. Therefore, the current study aimed to determine the effect of sleep quality on mtDNA-CN in eveningness chronotypes.

METHODOLOGY

A cross-sectional analytical study was conducted in the Department of Physiology, Islamic International Medical College (IIMC), Rawalpindi in collaboration with the Genetic Resource Centre. The study was approved by the Institutional Review Committee (IRC). The duration of study was ten months starting from August 2022 to May 2023. The sample size was calculated using the formula ($n = Z^2 (SD)^2 / e^2$). Inserting standard deviation of mitochondrial DNA copy number of 17.7 at 95% CI, the sample size was found to be 52.¹² However, 80 participants were included to improve the quality of the study. So, a total of 80 healthy eveningness chronotypes were recruited by circulating Morningness- Eveningness Questionnaire (MEQ) from Islamic International Medical College including students, faculty, and staff members.¹³ All participants were between 18 to 50 years of age to avoid any age related changes in mitochondrial DNA copy number.¹⁴ Smokers and individuals with any comorbidity (heart, liver, and kidney diseases) were excluded from the study. A written informed consent was taken from all participants of the study.

The sleep quality of all the participants was assessed by using Pittsburg Sleep Quality Index (PSQI). On the basis of PSQI, the participants were divided into 2 groups, Group A (good sleep quality) and Group B (poor sleep quality).¹⁵ The PSQI was a self-administered questionnaire that analyses the quality of sleep over the previous four weeks. It consisted of 19 self-reported questions with seven components: subjective sleep quality, sleep latency, sleep length, sleep efficiency, sleep disruption, and daytime dysfunction. Each component awarded a score ranging from zero to three, generating a PSQI score ranging from 0 to 21. A total score of 0 to 4 was regarded as good sleep

quality, whereas a number more than 4 was considered to be poor sleep quality. This had a diagnostic sensitivity and specificity of 89.6% and 86.5%, respectively.¹⁶

DNA was extracted from 2-3 ml peripheral blood using the Chelex method.¹⁷ The extracted DNA was stored at -20°C until qPCR amplification. An assay based on quantitative polymerase chain reaction using HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX), 5x was adapted as a measure of the amount of mitochondrial DNA copy number qPCR was used to quantify the copy number of the mitochondrial gene NADH dehydrogenase, subunit 1 (ND1). Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene was used as a reference for the nuclear DNA copy number. The real-time polymerase chain reactions (PCR) were carried out using Rotor-Gene Q by Qiagen to acquire the respective cycle thresholds (Ct) values for copy numbers of ND1 and CFTR (control) gene.

The primers were used to amplify nuclear and mitochondrial genes of interest:

MtDNA primers: (153 bp)¹⁴

MtDNA-F 5'-AACATACCCATGGCCAACCT-3'

MtDNA-R 5'-AGCGAAGGGTTGTAGTAGCCC-3'

Control (CFTR Gene) primers: (97 bp)

CFTR-F 5'-GTTTTCTGGATTATGCCTGGCAC-3'

CFTR-R 5'-GTTGGCATGCTTTGATGACGCTTC-3'

The PCR reaction was run in a tube containing forward and reverse primers specific to the gene. The final total volume of the PCR reaction was 20 micro-litres. PCR was run for both nuclear and mitochondrial DNA of each sample simultaneously. PCR was done by initial DNA denaturation at 95°C (2 min) accompanied by 35 cycles of 95°C for 20 seconds (denaturation), 60°C for 60 seconds (annealing, extension, and image acquisition). Relative mitochondrial DNA copy number was calculated by first calculating delta Ct through the formula: Delta Ct (ΔCt) = Ct nuclear gene - Ct mitochondrial DNA. After calculation of delta Ct, another formula was applied to get mitochondrial DNA copy number¹⁸ as mitochondrial DNA copy number = $2^{\Delta Ct}$.

The data were analysed through SPSS version 27, the mean \pm standard deviation of mitochondrial DNA copy number of both groups were compared by Independent sample t-test. The p-value ≤ 0.05 was considered statistically significant. The categorical variables were expressed as frequency and percentages.

RESULTS

In this cross-sectional study, a total of 80 participants with evening chronotypes based on Morningness-Eveningness Questionnaire (MEQ) were included, out of which, 37.5% (n=30) individuals were male and 62.5% (n=50) were females.

The mean age was 24.27 ± 6.91 years ranging from 18 to 45 years. The determination of mitochondrial DNA copy number in both groups were normally distributed when transformed to log values.

Table I: Comparison of MtDNA-CN in peripheral blood of Group A and B.

Group	Number	Mean	Range	SD
Group A (Good Sleep Quality)	40	2.65	2.39-2.91	± 0.26
Group B (Poor Sleep Quality)	40	2.34	2.05-3.24	± 0.29
p-value		<0.001		

Analysis was done by using Independent-samples t-test.

In Group A, 25 (62.5%) out of 40 participants were females and 15 (37.5%) were males. In Group B, there were 25 (62.5%) females and 15 (37.5%) males. Mitochondrial DNA copy number was not statistically significant with gender in both groups.

Similarly in Group A, 21 (52.5%) were faculty members and 19 (47.5%) were students, and in Group B, 30 (75%) were faculty members and 10 (25%) were students. However, mitochondrial DNA-CN was not significantly associated with professional status in both groups.

On comparison of the sleep quality, the mean \pm standard deviation mitochondrial DNA copy number in Group A (2.65 ± 0.26), exhibited significant increase (95% CI: 0.18, 0.43; $p < 0.001$) as compared to Group B (2.34 ± 0.29). The comparison is shown in Table I.

DISCUSSION

The growing burden of oxidative stress, has been linked to various age-related diseases such as non-communicable diseases (NCDs) and cancer. Mitochondrial DNA copy number has emerged as a potential biomarker for monitoring oxidative stress levels. There is a lack of cost-effective and easily accessible methods for early detection and monitoring of high-risk individuals in the general population. By implementing biomarker-based screening methods, healthcare professionals can better identify individuals who would benefit from targeted interventions and personalised preventive measures. This approach can ultimately lead to improved health outcomes, reduced healthcare costs, and a more efficient allocation of resources in the field of disease prevention and management.

The current study confirmed the positive effects of good sleep quality on mitochondrial DNA copy number in eveningness chronotypes. Mitochondrial DNA copy number (log transformed) of eveningness chronotype with good sleep quality was significantly higher as compared to those experiencing poor sleep quality.

The findings of the current study were consistent with those of Wrede *et al.*, who investigated the effect of sleep duration on mitochondrial DNA copy number in monozygotic twins. Wrede *et al.* claimed to be the first to discover such a relationship in human population. Shorter sleep duration and worse sleep efficiency were connected to a reduction in mitochondrial DNA copy number, according to their research.¹² The current study concentrated on sleep quality, taking into account both sleep length and efficiency as important factors.

Its findings also revealed a link between greater mitochondrial DNA copy number and better sleep quality, while lower mitochondrial DNA copy number was related with worse sleep quality. Unlike Lacedonia *et al.*'s study, which focused on individuals with obstructive sleep apnoea (OSA) and discovered higher mitochondrial DNA copy numbers in OSA patients compared to a control group, the current study specifically recruited healthy participants with no underlying sleep disorders.¹⁹

Shin *et al.* conducted a research to investigate the link between salivary mitochondrial DNA copy number (mtDNA-CN) and fatigue. Their findings revealed a negative relationship between chronic fatigue and mtDNA-CN, although the exact cause of fatigue was not established. Poor sleep quality was recognised as one of the potential contributors of fatigue.²⁰ The current study used whole blood samples to explore the effect of sleep quality on mtDNA-CN without assessing individuals' fatigue levels.

Ana *et al.* demonstrated that sleep deprivation in mice impaired the electron transport chain, resulting in mitochondrial dysfunction when compared to a control group.²¹ In the current study, the attention was shifted to human sleep quality, using mitochondrial DNA copy quantity as a measure of mitochondrial function.

The existing research found a correlation between smartphone addiction among students and poor sleep quality, resulting in insomnia. Furthermore, Al Bathashi *et al.* observed that smartphone addiction is linked to mental health issues such as anxiety and stress.²² While the current research did not directly target smartphone users, it recruited individuals with an eveningness chronotype who may or may not use smartphones. It is worth noting, however, that the psychological stress found in the Al Bathashi *et al.*'s study might be related to changes in mitochondrial DNA copy number (mtDNA-CN). Further research will be needed to investigate the potential link between mtDNA-CN alteration and psychological stress.

A related research found similar results, demonstrating that poor sleep quality relates to increased oxidative stress in those with coronary artery disease. In that investigation, lipid hydroperoxides and 8-isoprostane were used as biomarkers to assess levels of oxidative stress.²³ However, in the current investigation, mitochondrial DNA copy number was used as a biomarker of oxidative stress. The current study examined the possible influence of sleep on oxidative stress in research population by investigating the relationship between sleep quality and mitochondrial DNA copy number.

Kanagasabai *et al.* conducted a study that found that sleep deprivation and poor sleep quality had a detrimental effect on oxidative stress, antioxidant imbalance, and the formation of a pro-inflammatory state. These variables could raise the chance of getting diabetes.²⁴ In accordance with this, the present study supported the idea that poor sleep quality contributes to increased oxidative stress, as revealed by a decrease in mitochondrial DNA copy number among those who have an eveningness chronotype.

There were various limitations to the current study. First, the cross-sectional methodology used in this study only gave a snapshot of the association between sleep quality and mitochondrial function at a given point in time, making it unable to establish causative relationships or examine changes over time. Longitudinal study would be useful in unravelling temporal dynamics and determining cause-and-effect links in the future. Another issue was the dependence on self-report measures to determine sleep quality, which was susceptible to recall bias and lacked objective evaluation of sleep patterns. In future investigations, objective sleep quality measures such as actigraphy or polysomnography would give more exact and thorough data. Furthermore, the study's sample was limited to a specific population or setting, potentially limiting the generalisability of the findings. Future research should aim to include diverse samples to enhance external validity.

CONCLUSION

People with good sleep quality had a higher mtDNA-CN than those with poor sleep quality. Good sleep quality may counteract the negative effects of increased oxidative stress and ageing brought on by the eveningness behaviour, thus leading to better mitochondrial function and increased mitochondrial biogenesis indicated by higher mtDNA-CN in individuals who experience good sleep quality.

ETHICAL APPROVAL:

Before starting this research, an ethical approval was taken from the Institutional Review Committee (IRC) under reference number (Riphah/IIMC/IRC/22/2068).

PATIENTS' CONSENT:

All participants signed a written consent form to agree to publication of the data.

COMPETING INTEREST:

All authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MM: Design and drafting of research work, acquisition, compilation and analysis of data, financial resource, manuscript writing.

SA: Concept of research, supervision of manuscript-writing and conduction of experiment, review and final approval of the project.

SA: Facilitating the experimental procedure and data collection.

SF: Conceived the idea.

All authors agreed to publish the final version of the manuscript.

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