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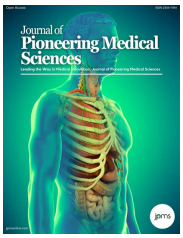

Kynurenine 3-Monooxygenase Protein and its Association on the Sleep Quality in Cigarette Smokers and Non-smokers

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Abstract **Objectives:** Background: Cigarette smoking significantly impacts sleep quality by altering neurochemical pathways. Nicotine, an agonist of nicotinic acetylcholine receptors, stimulates the release of dopamine, serotonin and norepinephrine, which can hinder the initiation and maintenance of sleep. This study focuses on the kynurenine pathway, where nicotine-induced Kynurenine 3-monooxygenase (KMO) activity leads to elevated levels of neurotoxic metabolites, such as quinolinic acid, that impair sleep quality in smokers. **Methods:** This cross-sectional study included 260 participants, equally divided into smokers and non-smokers, with informed consent obtained from all participants. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), covering parameters such as sleep latency, duration and disturbances. Saliva samples were collected and analyzed via ELISA to measure KMO biomarker levels. Statistical analyses, including Chi-Square and Mann-Whitney U tests, were employed to examine the relationship between KMO levels and sleep quality while acknowledging the limitations of self-reported data and potential confounders. **Results:** KMO levels were significantly higher in smokers (1.03 ± 0.13) compared to non-smokers (0.95 ± 0.09). Overall, 57.31% of participants reported good sleep, while 42.69% experienced poor sleep. Among smokers, 32.31% experienced poor sleep and only 17.69% had good sleep. Conversely, 39.62% of non-smokers reported good sleep, while 10.38% experienced poor sleep. Statistical analysis confirmed the association between smoking and disturbed sleep quality, though subgroup analyses by smoking intensity were not explored. **Conclusion:** The study confirms that smokers exhibit higher KMO levels and poorer sleep quality compared to non-smokers. These findings highlight the potential of targeting KMO levels as a therapeutic approach to mitigate sleep disturbances in smokers. Future research should explore pharmacological and behavioral interventions, while addressing broader public health implications and improving study methodologies.

Key Words Smokers, non-smokers, sleep quality, KMO, biomarkers, neurotoxic metabolites, nicotine dependency

INTRODUCTION

The quality of sleep plays a vital role in human health and well-being, influencing physical, mental and emotional states [1]. Sleep disturbances are increasingly recognized as critical factors contributing to various health conditions. Cigarette smoking, despite its well-documented harmful health impacts, stands out as a significant driver of poor sleep quality [2]. Smoking disrupts several physiological processes necessary to maintain regular sleep cycles [3] and increases the risk of conditions like rheumatoid arthritis, further compounding health challenges [4].

Nicotine, a potent stimulant, impacts the central nervous system by entering the bloodstream through cigarette smoking [5]. Smokers may find it challenging to fall and remain asleep due to neurochemical changes that elevate alertness and diminish the perceived need for sleep [6]. Studies consistently show that smokers experience lower-quality sleep compared to non-smokers [7]. Research highlights reduced sleep efficiency, shorter sleep duration and less time spent in restorative phases such as slow-wave and REM sleep among smokers [8]. Despite spending comparable total time in bed, smokers often experience fragmented and less restorative sleep than non-smokers [9].

Kynurenine 3-monooxygenase (KMO) is a key enzyme in the kynurenine pathway, a major route for tryptophan metabolism [10]. This pathway produces numerous

metabolites, some of which are neuroactive and can significantly influence mood, cognitive functions and sleep quality. Elevated KMO activity, often stimulated by nicotine exposure, has been linked to increased levels of neurotoxic metabolites like quinolinic acid, which negatively impact sleep. Conversely, kynurenic acid, known for its neuroprotective and anti-inflammatory properties, is associated with improved sleep quality [11,12].

Emerging evidence suggests that smoking alters the kynurenine pathway by increasing KMO activity and disrupting the balance of its downstream metabolites. The heightened production of neurotoxic metabolites contributes to the poor sleep quality frequently observed in smokers. Inhibiting the KMO enzyme has shown promise in elevating kynurenic acid levels, offering potential therapeutic benefits for managing tobacco dependence and related sleep disturbances [11]. This underscores the kynurenine pathway's critical role in understanding and addressing the interplay between smoking and sleep quality.

Previous studies by Cohrs *et al.* [13] evaluated sleep quality and duration in smokers, while Zhang *et al.* [14] analyzed the impact of smoking on sleep architecture, particularly REM and non-REM sleep phases. However, there remains a gap in understanding the specific association between KMO activity and sleep disturbances in smokers.

This study aims to compare the sleep quality between smokers and non-smokers and to explore the correlation between KMO levels and sleep disturbances in these two groups. By examining these associations, this research seeks to contribute to the growing understanding of the biochemical pathways involved in smoking-related sleep disorders and their potential implications for treatment.

METHODS

Study Design and Sample Size

This cross-sectional study included a sample of 260 participants, equally divided into smokers (n = 130) and non-smokers (n = 130). The sample size was determined using a pilot study. Informed consent was obtained from all participants, ensuring ethical compliance. Participants were recruited based on defined inclusion and exclusion criteria, although these were not detailed here and warrant clarification in future studies.

Data Collection

Saliva samples were collected from all participants using sterile saliva-collecting containers. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire, which evaluates components such as sleep latency, subjective sleep quality, sleep duration, disturbances, habitual sleep efficiency, daytime dysfunction and use of sleeping medication. Each component is scored from 0 to 3, with a total score ranging from 0 to 21. Scores of 0-5 denote good sleep quality, while scores $\geq$6 indicate poor sleep quality and potential sleep disorders. To minimize recall bias, participants were given clear instructions on completing the PSQI questionnaire.

Saliva Sample Analysis

Saliva samples were analyzed for Kynurenine 3-Monooxygenase (KMO) biomarker levels using an enzyme-linked immunosorbent assay (ELISA). The detailed ELISA procedure included the following steps:

- **Preparation of Standards and Samples:** Standard, pilot sample and blank control wells were placed on the pre-coated ELISA plate. Positions were recorded for consistency. Each standard well received 100 µL of solutions from the zero, first, second, third and fourth tubes. A sample dilution buffer was added to the blank control wells. Each test well received 100 µL of saliva samples.
- **Incubation and Washing:** Plates were sealed and statically incubated for 90 minutes at 37°C. After incubation, the plate was tapped on absorbent paper to remove liquids. Without submersion, 350 µL of wash buffer was added to each well and discarded after one minute. The wells were washed twice, ensuring thorough cleaning.
- **Addition of Biotin-Labeled Antibody:** Each well received 100 µL of biotin-labeled antibody working solution. Plates were sealed and incubated at 37°C for 60 minutes. After incubation, the liquid was removed and wells were washed three times with 350 µL of wash buffer.
- **Addition of HRP-Streptavidin Conjugate:** To every well, 100 µL of HRP-Streptavidin Conjugate SABC working solution was added. Plates were sealed and incubated at 37°C for 30 minutes. Wells were washed five times with wash buffer to ensure removal of unbound substances.
- **Color Development and Optical Density (O.D.) Measurement:** After adding 90 µL of TMB substrate solution, plates were incubated statically for 10-20 minutes at 37°C in the dark. To stop the reaction, 50 µL of stop solution was added to each well, immediately turning the liquid yellow. O.D. absorbance was measured at 450 nm using a microplate reader.

Statistical Analysis

The collected data were subjected to statistical analysis using the Chi-Square test and Mann-Whitney U test. Descriptive statistics were used to summarize the findings. P-values and Z-values were computed to evaluate the significance of results, which were tabulated for comparison between smokers and non-smokers. However, potential confounding factors such as alcohol use, medication and lifestyle variables were not accounted for in this study, representing a limitation.

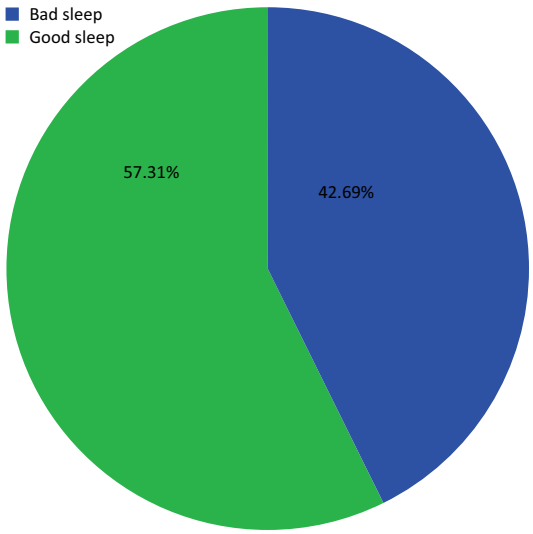

Figure 1: Pie chart showing sleep quality among study participants

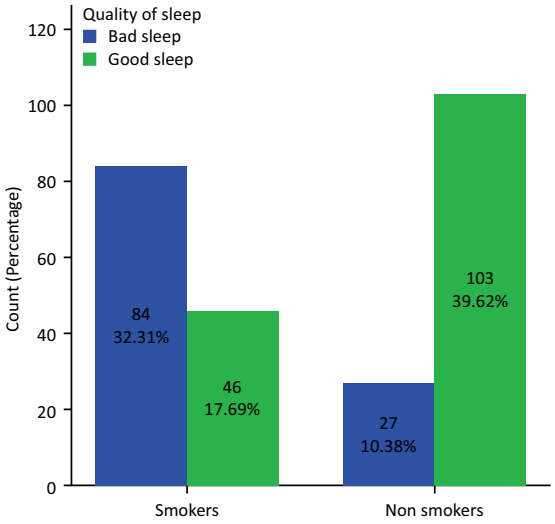

Figure 2: Bar chart showing quality of sleep among smokers and non smokers

RESULTS

A cross-sectional study was conducted among 260 outpatients in a dental college to assess the relationship between sleep quality and Kynurenine 3-Monooxygenase (KMO) biomarker levels among smokers and non-smokers. The findings are presented below:

Sleep Quality Among Study Participants

The pie chart (Figure 1) illustrates the proportion of participants reporting good and poor sleep quality. Overall, 57.31% of individuals reported having good sleep, while 42.69% experienced poor sleep. The majority of participants demonstrated good sleep quality, highlighting variability across the groups.

Comparison of Sleep Quality Between Smokers and Non-Smokers

The bar chart (Figure 2) compares the distribution of sleep quality between smokers and non-smokers. Among smokers, 32.31% (84 individuals) reported experiencing poor sleep, while only 17.69% (46 individuals) reported good sleep. In contrast, among non-smokers, a larger proportion (39.62%, 103 individuals) reported good sleep, while a smaller proportion (10.38%, 27 individuals) reported poor sleep. These findings indicate that non-smokers generally experience better sleep quality compared to smokers. The statistical analysis showed a significant association between smoking status and sleep quality (Chi-Square value = 51.08, $p<0.001$) (Figure 2).

KMO Levels Among Smokers and Non-Smokers

Table 1 shows that smokers had significantly higher KMO levels (1.03±0.13) compared to non-smokers (0.95±0.09). This finding suggests that smoking is associated with elevated KMO biomarker levels, which may contribute to disrupted sleep patterns.

Table 1: KMO values among smokers and non smokers

	KMO (ng/mL)			
	N	Mean	Standard Deviation	Standard Error mean
Smokers	130	1.034485	0.129486	0.011357
Non smokers	130	0.952369	0.091190	0.007998

Table 2: Mann Whitney U Test Results Regarding the KMO levels and sleep quality

	KMO				
Sleep quality	N	Mean Rank	U	Z	P
Bad sleep	111	129	8436.5000	0.279	0.780
Good sleep	149	131.62			

Table 3: Mann Whitney U Test Results Regarding the KMO levels among smokers and nonsmokers. The mean rank value for smokers is 152.53 and non smokers is 108.47

	KMO				
	N	Mean Rank	U	Z	P
Smokers	130	152.53	5586.5	-4.731	0.000
Non smokers	130	108.47			

Association Between KMO Levels and Sleep Quality

Using the Mann-Whitney U Test, the mean rank for participants with poor sleep quality was 129, while for those with good sleep quality, it was 131.62. These results indicate that the difference in KMO levels between participants with good and poor sleep quality was minimal and statistically insignificant ($U = 8436.5000$, $p = 0.780$) (Table 2).

Correlation Between KMO Levels and Smoking Status

The analysis revealed a significant difference in KMO levels between smokers and non-smokers ($U = 5586.5$, $Z = -4.731$,

p<0.001) (Table 3). This finding emphasizes the strong relationship between smoking and increased KMO activity, further supporting its potential role in sleep disturbances among smokers.

DISCUSSION

Smoking remains a leading cause of preventable diseases and deaths, significantly impacting both smokers and individuals exposed to secondhand smoke. The harmful substances in tobacco, including nicotine, tar and carbon monoxide, heighten the risks of cancer, cardiovascular disease, respiratory conditions and immune suppression [15,16]. While the immediate and long-term health benefits of quitting smoking-such as improved pulmonary function and reduced risks of chronic illnesses-are well-documented, smoking's impact on sleep quality is less understood but equally crucial. Good sleep quality is a fundamental component of physical and mental health. Cigarette smoking is a key disruptor of sleep architecture, with nicotine acting as a stimulant that alters sleep patterns. Smokers often report delayed sleep onset and fragmented sleep due to nicotine's stimulating effects on the central nervous system [17,18]. In this study, the evaluation of smokers' and non-smokers' sleep quality through the Pittsburgh Sleep Quality Index (PSQI) revealed significant disparities. Smokers exhibited higher Kynurenine 3-Monooxygenase (KMO) levels compared to non-smokers, which was associated with poor sleep quality. This finding supports prior research indicating that nicotine exposure increases KMO activity, leading to the production of neurotoxic metabolites such as quinolinic acid, which negatively impacts sleep [19].

The dose-response relationship between smoking intensity and the prevalence of sleep disturbances aligns with prior findings. For instance, Cohrs *et al.* [13] observed that nicotine withdrawal symptoms and stimulant effects reduce sleep duration by approximately 14 minutes per night among smokers compared to non-smokers. Similarly, Zhang *et al.* [14] reported reduced sleep efficiency and increased daytime fatigue in smokers. Studies also link smoking cravings and quitting attempts with higher rates of sleep disturbances [9]. Frequent awakenings and greater sleep fragmentation are further exacerbated by nocturnal nicotine withdrawal, as noted by Phillips [20]. These disruptions collectively contribute to the lower sleep quality observed in smokers.

From a biochemical perspective, the heightened activity of the kynurenine pathway in smokers suggests a crucial role for KMO in mediating the adverse effects of smoking on sleep. Schwarcz et al. demonstrated that lower KMO activity in non-smokers may enhance sleep architecture by increasing time spent in restorative sleep phases and minimizing disturbances [21]. This study's findings further confirm that elevated KMO activity in smokers contributes to higher levels of neurotoxic metabolites, reinforcing the link between KMO and poor sleep quality.

Tobacco use, whether through smoking or chewing, also increases the risk of oral potentially malignant disorders (OPMDs) and oral cancer, emphasizing the broad health implications of tobacco exposure [22,23]. Public health measures-including education, awareness campaigns and smoking cessation support-are critical for reducing the societal and healthcare burdens associated with smoking [24,25].

Limitations and Future Directions

While this study sheds light on the relationship between KMO levels, smoking and sleep quality, several limitations must be acknowledged. Self-reported data on sleep patterns can introduce recall bias, compromising the accuracy of findings. Furthermore, the inability to control for confounding factors such as alcohol consumption, medication use, stress levels and diet limits the generalizability of the results. The cross-sectional design of this study precludes establishing causal relationships, which future longitudinal research should address.

Future studies should also evaluate interventions aimed at modulating KMO activity, either through pharmacological agents or lifestyle modifications, to improve sleep quality among smokers. Investigating gender-specific and cultural differences in smoking behaviors and sleep patterns could provide a more comprehensive understanding. Additionally, incorporating objective sleep assessments, such as polysomnography and exploring other biochemical pathways could enhance the robustness of findings.

CONCLUSION

This study highlights that smokers exhibit significantly poorer sleep quality compared to non-smokers, primarily due to elevated KMO levels. These elevated KMO levels, driven by nicotine exposure, promote the production of neurotoxic metabolites, which disrupt sleep architecture. Targeting KMO activity may offer a promising therapeutic avenue for addressing smoking-induced sleep disturbances. Integrating these findings into smoking cessation programs and public health strategies could significantly improve both sleep quality and overall health outcomes in smokers. Further research is essential to refine diagnostic tools and develop effective interventions for mitigating the adverse effects of smoking on sleep.

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Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this article. The study was conducted independently, with no influence from external parties or organizations.

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REFERENCES

[1] Purani, Himal, *et al.* "Sleep quality in cigarette smokers: Associations with smoking-related outcomes and exercise." *Addictive Behaviors,* vol. 90, March 2019, pp. 71-76. http://dx.doi.org/10.1016/j.addbeh.2018.10.023.

[2] Frange, C. *Clinical Cases in Sleep Physical Therapy*. Springer Nature, 2023, Pages: 300.

[3] Wetter, D.W. and T.B. Young. "The relation between cigarette smoking and sleep disturbance." Preventive Medicine, vol. 23, no. 3, May 1994, pp. 328-334. http://dx.doi.org/10.1006/pmed.1994.1046.

[4] Sachin, Aditya B. *et al.* "Knowledge, prevalence and risk factors of rheumatoid arthritis among middle-aged and old aged population." *International Journal of Research in Pharmaceutical Sciences*. Vol. 7, no. 11, October 2020, pp. 522-528. https://ijrps.com/home/article/view/2546/9707.

[5] Hall, F. Scott, *et al.* "Negative affective states and cognitive impairments in nicotine dependence." *Neuroscience and Biobehavioral Reviews*, vol. 58, November 2015, pp. 168-185. http://dx.doi.org/10.1016/j.neubiorev.2015.06.004.

[6] Grandner, Michael A. *Sleep and Health*. Academic Press, 2019, Pages: 534. https://www.sciencedirect.com/book/9780128153734/sleep-and-health.

[7] CDC. *Preventing Tobacco Use Among Youth and Young Adults: A Report of the Surgeon General*. Centers for Disease Control and Prevention, 2012, Pages: 22. https://www.cdc.gov/tobacco/sgr/2012/consumer_booklet/pdfs/consumer.pdf.

[8] Silva, Wennyo Camilo da Silva e, *et al.* "Sleep quality of adult tobacco users: A systematic review of literature and meta-analysis." *Sleep Epidemiology,* vol. 2, no. 2022, December 2022,. http://dx.doi.org/10.1016/j.sleepe.2022.100028.

[9] Liao, Yanhui, *et al.* "Sleep quality in cigarette smokers and nonsmokers: Findings from the general population in central China." *BMC Public Health*, vol. 19, no. 1, June 2019,. http://dx.doi.org/10.1186/s12889-019-6929-4.

[10] Deatley, Teresa, *et al.* "Psychometric analysis of a microenvironment secondhand smoke exposure questionnaire." *International Journal of Environmental Research and Public Health,* vol. 18, no. 7, April 2021,. http://dx.doi.org/10.3390/ijerph18073753.

[11] Aziz, Hassan A. *et al.* "Kynurenine 3-monooxygenase gene associated with nicotine initiation and addiction: Analysis of novel regulatory features at 5 and 3 -regions." *Frontiers in Genetics,* vol. 9, June 2018,. http://dx.doi.org/10.3389/fgene.2018.00198.

[12] Savitz, Jonathan. "The kynurenine pathway: A finger in every pie." *Molecular Psychiatry,* vol. 25, no. 1, April 2019, pp. 131-147. http://dx.doi.org/10.1038/s41380-019-0414-4.

[13] Cohrs, Stefan, *et al.* "Impaired sleep quality and sleep duration in smokers-results from the German Multicenter Study on Nicotine Dependence." *Addiction Biology,* vol. 19, no. 3, August 2012, pp. 486-496. http://dx.doi.org/10.1111/j.1369-1600.2012.00487.x.

[14] Zhang, Lin, *et al.* "Cigarette smoking and nocturnal sleep architecture." *American Journal of Epidemiology*, vol. 164, no. 6, July 2006, pp. 529-537. http://dx.doi.org/10.1093/aje/kwj231.

[15] Nivethitha, R. and L. Leelavathi. "Awareness on ill effects of tobacco usage among tobacco users." *Journal of Advanced Pharmaceutical Technology and Research*, vol. 13, no. 1, 2022. 10.4103/japtr.japtr_147_22 pp: S217-S222. http://dx.doi.org/10.4103/japtr.japtr_147_22

[16] Ahmed, Naveed, *et al.* 2021. Smoking a dangerous addiction: A systematic review on an underrated risk factor for oral diseases. *International Journal of Environmental Research and Public Health*, Vol. 18, No. 21,. 10.3390/ijerph182111003. http://dx.doi.org/10.3390/ijerph182111003.

[17] Jaehne, Andreas, *et al.* "How smoking affects sleep: A polysomnographical analysis." *Sleep Medicine,* vol. 13, no. 10, December 2012, pp. 1286-1292. http://dx.doi.org/10.1016/j.sleep.2012.06.026.

[18] Patterson, Freda, *et al.* "Sleep as a target for optimized response to smoking cessation treatment." *Nicotine and Tobacco Research,* vol. 21, no. 2, October 2017, pp. 139-148. http://dx.doi.org/10.1093/ntr/ntx236.

[19] Sun, Haoxiong and Sijia Li. "Exploring the relationship between smoking and poor sleep quality: A cross-sectional study using NHANES." *Frontiers in Psychiatry,* vol. 15, May 2024,. http://dx.doi.org/10.3389/fpsyt.2024.1407741.

[20] Phillips, Barbara A. "Cigarette smoking and sleep disturbance." *Archives of Internal Medicine,* vol. 155, no. 7, April 1995, pp. 734-737. http://dx.doi.org/10.1001/archinte.1995.00430070088011.

[21] Schwarcz, Robert, *et al.* "Kynurenines in the mammalian brain: When physiology meets pathology." *Nature Reviews Neuroscience,* vol. 13, no. 7, June 2012, pp. 465-477. http://dx.doi.org/10.1038/nrn3257.

[22] Ep, Raguladhithya. "*Inflammation in smokers and non-smokers during implant surgery among Indians.*" Bioinformation, vol. 19, no. 4, April 2023, pp. 510-513. https://pubmed.ncbi.nlm.nih.gov/37822839.

[23] Antony, J.Vini Mary, *et al.* 2021. Particle size, penetration rate and effects of smoke and smokeless tobacco products-an *in vitro* analysis. Heliyon, Vol. 7, No. 3,. 10.1016/j.heliyon.2021.e06455. http://dx.doi.org/10.1016/j.heliyon.2021.e06455.

[24] Ramalingam, Karthikeyan, *et al.* 2023. Assessment of oral lesions with tobacco usage: A cross-sectional clinicopathological study in Sri Ganganagar, Rajasthan, India. Cureus, Vol. 15, No. 1,. 10.7759/cureus.33428. http://dx.doi.org/10.7759/cureus.33428.

[25] Chellappa, Lalitha Rani, *et al.* "A qualitative study on attitude towards smoking, quitting and tobacco control policies among current smokers of different socio-economic status." Medknow, *Journal of Family Medicine and Primary Care,* vol. 10, no. 3, March 2021, pp. 1282-1287. http://dx.doi.org/10.4103/jfmpc.jfmpc_1628_20.