Original Article

To determine melatonin, cytokines, and sleep index in type 2 diabetes mellitus individuals

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Abstract

Introduction: The pathogenesis of type 2 diabetes mellitus (T2DM) is influenced by inflammation and oxidative stress. People with T2DM show evidence of sleep disruption, and their melatonin rhythm, which regulates sleep, is aberrant. It is still uncertain, nevertheless, whether inflammation in this group contributes to the inhibition of melatonin synthesis and sleep problems. Hence, the study aimed to correlate and characterize the biological variables of melatonin levels, inflammatory cytokine levels, and sleep parameters in patients with T2DM. **Material and Methods:** ELISA was used to analyze melatonin and cytokine levels in blood samples, and the Pittsburgh Sleep Quality Index (PSQI) questionnaire was utilized to determine sleep quality. **Results:** In the global sleep quality measure (PSQI questionnaire), the control group did better than the T2DM group, indicating lower sleep quality and a greater incidence of sleep problems. Melatonin production lacked rhythmicity and was lower in patients with T2DM than in controls both during the day and at night. The T2DM group showed greater levels of chemerin, IL-1, and a negative connection between melatonin and chemerin levels than the control group. **Conclusion:** The results suggest that the low melatonin production seen in the T2DM group was most likely the underlying cause of the sleep pathology seen there. It is most probable that high levels of chemerin, which have been linked to other pathologies in the past, are to blame for the blocking of melatonin production in T2DM.

Keywords: Chemerin, circadian rhythm, interleukin-1, melatonin, Pittsburgh sleep quality index questionnaire, sleep

Introduction

Type 2 diabetes mellitus (T2DM) affects the structural and functional units of life that consequently produce inflammatory cytokines and hormones due to biochemical changes caused by pathological glucose levels.[1] T2DM research reported that etiology of the onset, development, and complications are influenced by inflammation and oxidative stress.[2] As a result, individuals with T2DM frequently have elevated levels of inflammatory mediators in their blood, including tumor necrosis factor, chemerin, interleukin 1 (IL-1), and IL-4, as well as oxidative stress markers.[3-5] T2DM patients typically report sleep issues,[6-8] which have been connected to a potential inhibition of the nocturnal elevate in melatonin levels, along with this inflammatory disease.[9-11]

The mechanism for melatonin production includes the signaling of ambient light-dark alternation to the pineal gland. The pineal gland's arylalkyl-amine-N-acetyltransferase converts serotonin to N-acetyl-serotonin, a precursor that is subsequently methylated by acetyl-serotonin O-methyltransferase to create 5-methoxy-

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N-acetyltriptamine or melatonin when light is absent.[12] It is well known that melatonin secretion peaks at around 3:00 a.m. when there is the highest concentration.[11] To detect the darkness in its surroundings, the body increases nocturnal melatonin secretion and releases melatonin into the blood and cerebrospinal fluid. In addition to serving as an anti-inflammatory molecule, acquired immune responses and a regulator of innate, a catalyst for dendritic stability, and a neuroprotective molecule, melatonin also detects environmental circadian information in the body and regulates numerous biological rhythms, such as the body temperature, sleep-wake cycle, and neuroendocrine responses.[13,14]

In contrast, pathological conditions can alter melatonin production. Pathogen-associated signaling molecules and pro-inflammatory cytokines have previously been shown to inhibit the pineal gland's ability to synthesize this hormone, thus there is an untested hypothesis in T2DM that inflammatory molecules may block melatonin production.[15]

Understanding the control and dynamics of melatonin synthesis under pathological and physiological ailments is vital because of the vast spectrum of positive benefits of melatonin. In light of the potential association between inflammatory conditions, melatonin blockade, and sleep disturbances in T2DM, the study aimed to correlate and characterize the biological variables of melatonin levels, inflammatory cytokine levels, and sleep parameters in patients with T2DM.

Materials and Methods

This clinical cross-sectional investigation was carried out in compliance with the laws governing human research. The study protocol was certified by the institution's ethics committee (MU/Research/EC/Ph.D / 2019 / 014(a). Before beginning the study, each participant provided informed consent. Individuals were aged <50 years and those with pathological conditions were excluded. The inclusion criteria for healthy controls were not having T2DM, not using dietary supplements, being at least 50 years old, and having no known pathological conditions. The inclusion criteria for the T2DM group were T2DM for more than eight years, and the existence of neurological or mental problems acted as the exclusion criterion for both groups as well as the use of substances that affect melatonin production and/or release, such as melatonin, psychoactive drugs, or other medicines. Participants aged 52–77 (63.9 ± 2.4 years) were separated into two groups: 110 individuals with T2DM and 110 individuals without diabetes. None of the participants showed evidence of obesity or sleep apnea syndrome.

Slumber disturbances were gauged with the PSQI questionnaire. This is a one-month self-report questionnaire that examines sleep-related symptoms. It collects data on sleep quality and sleep disturbances. Nineteen self-reported items were divided into five subscales: subjective sleep quality, sleep duration, sleep medications, habitual sleep efficiency, and sleep disturbances. Each subscale score indicates the severity of sleep apnea. Lower scores indicated better sleep quality. A PSQI score of 5 or higher indicates significant or moderate difficulty with at least two or more sleep quality aspects.[16]

Blood samples, parameters analyzed, and instruments used

Serum chemerin and IL-1 levels were assessed using an ELISA kit purchased from Invitrogen Laboratories. Serum melatonin was assessed using the Elisa assay method purchased from Eagle Biosciences Laboratories. Our study's major shortcoming is that we neglected to take into account melatonin's circadian rhythm. Therefore, the authors of this study attempted to compensate for this shortcoming by taking blood samples from all subjects simultaneously. A ROBONIK microplate reader was used to measure ELISA assay readings. The reagents were mixed according to the instructions provided in the kit manual of the respective parameters. Under strict sterile conditions, 5 ml of fasting venous blood was drawn from each subject in both groups and collected in plane vials with a disposable syringe and needle. Serum samples were aliquoted after the blood had been separated by centrifugation at 3000rpm for 20 min. They were then kept at 20 degrees Centigrade until the test.

Statistical analysis

The mean standard error is shown for all values. A statistical *t*-test between two groups was used to assess the data. The correlation between the two factors was investigated using Pearson's correlation coefficient. The correlation between independent and dependent factors was determined using scattered regression coefficients. The cutoff for statistical significance was chosen at $P < 0.05$.

Results

The global mean PSQI score for those with T2DM was 7.3 ± 33.3, whereas it was just 3.91 ± 1.1 for those without the disease, which means that the T2DM group slept less well [Table 1 and Figure 1]. In addition, 81.81% more people in the T2DM group had sleep problems than those in the control group (62.72%) [Figure 1].

Serum melatonin levels showed that individuals with T2DM had less melatonin than those in the control group [Figure 2]. The people with T2DM had a mean ± SD of 17.6 ± 3.9 pg/mL of melatonin, while the people who didn't have diabetes had a mean ± SD of 29.5 ± 4.3 pg/mL melatonin levels.

Table 1: Sleep quality in T2DM group and control group individuals. Mean±standard error of the mean Pittsburgh Sleep Quality Index score in the control (*n*=110) and T2DM (*n*=110) groups

Groups	PSQI Score	*P* value
Control (*n*=110)	3.9±0.3	<0.05
T2DM (*n*=110)	7.3±0.9	

Chemerin levels in the plasma were significantly higher in the T2DM group (99.6 ± 10.2 pg/mL) than in the control group (69.8 ± 8.1 pg/mL) [Figure 3]. A negative association was found between chemerin and melatonin levels ($R = -482$; $P = 0.05$). In persons with T2DM, low melatonin content was seen both in those with high IL-1 concentrations and those with low IL-1 concentrations [Figure 4]. Statistical analysis revealed no evidence of a link between the two variables. This was also the case for melatonin and chemerin.

Discussion

The recent research found that 90% of diabetic patients suffer from some kind of sleep disruption, which is much greater than the incidence of sleep disturbance earlier documented in other illnesses (59% in age- and sex-matched control participants). Sleep quality affects vitality and morbidity and therefore plays a crucial role in overall health.[17,18] The fact that the diabetes complication of insomnia affects many people underscores its seriousness. Previous research on other conditions has also shown that sleep disorders cause daytime sleepiness and problems with many functions such as the immune system and heart health.[17,19,20]

This study did not investigate the existence of sleep pathology before or after the development of T2DM, but the current study illustrates that people with T2DM had a higher frequency of this condition than the general population. In addition, existing research has shown a causal relationship between diabetes and sleep pathology through various potential pathological mechanisms. According to this theory, T2DM may cause sleep pathology by altering chemoreflex responsiveness, narrowing blood vessels due to oxidative stress, and accumulating various toxins. Vascular permeability loss is accelerated by sleep pathology because chronic intermittent hypoxia damages tissues through hypercoagulability and systemic hypertension, which in turn negatively affects renal, cardiac, and cerebral functions.[21-27]

Daytime drowsiness was the most frequent issue, followed by fragmented sleep, delayed sleep, extremely early awakening, sleep latency, nocturnal insomnia, and shorter sleep durations.[24,25,28,29]

Two potential causes of sleep disturbances include diabetes mellitus[15] and a lack of a typical nocturnal rise in melatonin content.[4,5] T2DM inhibits both diurnal and nocturnal melatonin production, resulting in irregular melatonin concentration. The serum melatonin concentration in the control group was greater than that in the T2DM group, consistent with previous studies.[11,14,30,31] Since hypnotics are routinely used in this demographic but have not been proved to improve sleep quality, melatonin may be an alternative for this group of patients with diabetes.[32]

Since melatonin is known to play a role in cellular processes like oxidative stress reduction,[12] nuclear translocation of transcription factors inhibition,[14] and direct regulation of several enzyme actions, antagonism, and intra-mitochondrial action, the results of this study point to additional factors that should be taken into consideration when determining what causes the obstruction of melatonin production in T2DM.

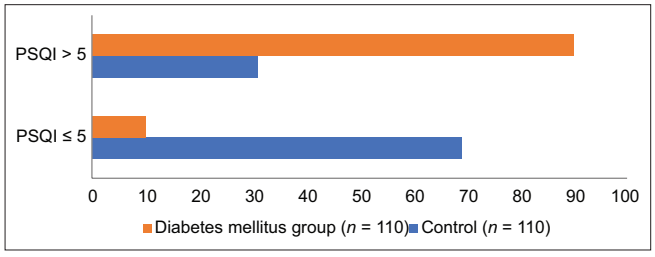

Figure 1: The Pittsburgh Sleep Quality Index was used to calculate the percentages of people with and without sleep disorders: Control subjects without sleep disorders (PSQI ≤ 5), control subjects with sleep disorders (PSQI >5), T2DM subjects without sleep disorders (PSQI ≤ 5) and T2DM subjects with sleep disorders (PSQI >5)

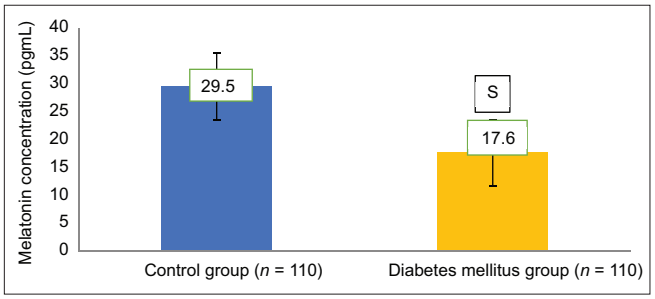

Figure 2: Comparison of serum melatonin levels between the control ($n = 110$) and T2DM ($n = 110$) groups, S = $P < 0.05$

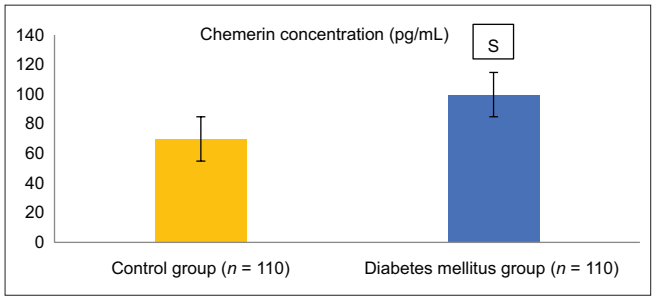

Figure 3: Comparison of serum chemerin levels between the control ($n = 110$) and T2DM ($n = 110$) groups, S = $P < 0.05$

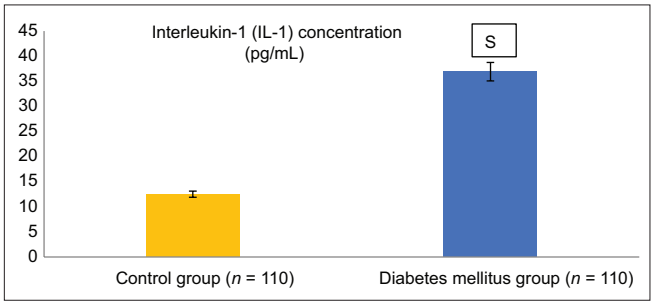

Figure 4: Comparison of serum interleukin-1 levels between the control ($n = 110$) and T2DM ($n = 110$) groups, S = $P < 0.05$

Melatonin is a neuroprotective molecule with anti-inflammatory characteristics,[14] modulates innate and acquired immunological responses,[15] has endocrine and metabolic activities in peripheral tissues,[29] and promotes dendritic stability.[33]

Patients with T2DM had higher plasma levels of the inflammatory mediators' chemerin and IL-1, according to prior results that were supported by the examination of cytokine concentrations in the present investigation.[34]

This is the first research to investigate whether elevated levels of inflammatory cytokines in people with T2DM may block the secretion of pineal gland melatonin, despite the existence of disease previously, models have shown that peripheral inflammation can do so.[35]

According to the current study, high chemerin levels in patients with T2DM were inversely related to low melatonin levels in this population. In addition to the fact that this correlation does not imply causation, this finding suggests that chemerin, as demonstrated in other conditions, may inhibit melatonin secretion in the pineal gland in T2DM. This supports the pineal-immune axis, which has been shown in various inflammatory illnesses.[11,15] According to this idea, under pathological circumstances, the immune system controls the production and release of melatonin from the pineal gland, which has an impact on the immune system. The nocturnal peak of melatonin is suppressed by the cytokine chemerin in the early stages of inflammation as a result of this complex and dynamic network that integrates signaling pathways and regulatory mechanisms at the cellular, molecular, as well as organismal levels.[13,14]

These results open the door to the creation of novel therapeutic approaches to raise physiological melatonin levels and address several melatonin-related diseases. These results might improve diabetic patient's quality of life by improving their sleep, which would also improve their quality of life.

Conclusion

These findings imply that the sleep issues seen in the diabetic group were likely caused by the reduced melatonin synthesis seen in this group. As has been shown in other illnesses, T2DM is likely caused by high levels of chemerin blocking the generation of melatonin. To enhance the quality of sleep and prevent non-chronobiotic activities of melatonin from being disrupted, this study supports future research on pharmacological methods to restore melatonin rhythm.

Compliance with ethical standards

The 1964 Helsinki Declaration and its subsequent revisions or equivalent ethical standards were followed in all procedures performed in studies involving human subjects. Additionally, these practices comply with institutional, national, and/or research committee ethical requirements.

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Authors' contributions

F.M conceived the manuscript and D.Z revised it. F.M, L.J.L, A.C, D.Z, P.K, F.N.K.Y and Q.M done the statistical analysis, F.M, L.J.L, A.C, D.Z, P.K, F.N.K.Y and Q.M wrote the manuscript. F.M, L.J.L, A.C, D.Z, P.K, F.N.K.Y and Q.M prepared tables and figures. Supervision was done by P.K. and D.Z. All authors have read and approved the manuscript.

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Conflicts of interest

There are no conflicts of interest.

References

1. Boarescu PM, Boarescu I, Pop RM, Roșian ȘH, Bocșan IC, Rus V, *et al.* Evaluation of oxidative stress biomarkers, pro-inflammatory cytokines, and histological changes in experimental hypertension, dyslipidemia, and type 1 T2DM. Int J Mol Sci 2022;23:1438.
2. Miettinen T, Sverloff J, Lappalainen OP, Linton SJ, Sipilä K, Kalso E. Sleep problems in pain patients entering tertiary pain care: The role of pain-related anxiety, medication use, self-reported diseases, and sleep disorders. Pain 2022;163:e812.
3. Bahnasy WS, El-Heneedy YA, El-Seidy EA, Labib NA, Ibrahim IS. Sleep disturbances in diabetic peripheral neuropathy patients: A clinical and polysomnographic study. Egypt J Neurol Psychiatr Neurosurg 2018;54:1-7.
4. Naranjo C, Del Reguero L, Moratalla G, Hercberg M, Valenzuela M, Failde I. Anxiety, depression and sleep disorders in patients with diabetic neuropathic pain: A systematic review. Expert Rev Neurother 2019;19:1201-9.
5. Rami AZ, Hamid AA, Anuar NN, Aminuddin A, Ugusman A. Exploring the relationship of perivascular adipose tissue inflammation and the development of vascular pathologies. Mediators Inflamm 2022;2022:2734321.
6. Khandelwal D, Dutta D, Chittawar S, Kalra S. Sleep disorders in type 2 diabetes. Indian J Endocrinol Metab 2017;21:758-61.
7. Naranjo C, Del Reguero L, Moratalla G, Hercberg M, Valenzuela M, Failde I. Anxiety, depression and sleep disorders in patients with diabetic neuropathic pain: A systematic review. Expert Rev Neurother 2019;19:1201-9.
8. Bener A, Al-Hamaq AO, Agan AF, Öztürk M, Ömer A. Sleeping disturbances and predictor risk factors among type 2 diabetic mellitus patients. Ann Afr Med 2020;19:230-6.
9. Brzecka A, Madetko N, Nikolenko VN, Ashraf GM, Ejma M, Leszek J, *et al.* Sleep disturbances and cognitive impairment in the course of type 2 diabetes-A possible link. Cur Neuropharmacol 2021;19:78-91.
10. Song SO, He K, Narla RR, Kang HG, Ryu HU, Boyko EJ. Metabolic consequences of obstructive sleep apnea especially pertaining to T2DM and insulin sensitivity.

Diabetes Metab J 2019;43:144-55.

11. Wajid F, Poolacherla R, Mim FK, Bangash A, Rutkofsky IH. Therapeutic potential of melatonin as a chronobiotic and cytoprotective agent in T2DM. J Diabetes Metab Disord 2020;19:1797-825.
12. Zhao D, Yu Y, Shen Y, Liu Q, Zhao Z, Sharma R, *et al.* Melatonin synthesis and function: Evolutionary history in animals and plants. Front Endocrinol (Lausanne) 2019;10:249.
13. Maestroni GJ, Conti A. Melatonin in relation to the immune system. In: Melatonin. CRC Press; 2020. p. 289-309.
14. Bondy SC, Campbell A. Melatonin and regulation of immune function: Impact on numerous diseases. Curr Aging Sci 2020;13:92-101.
15. Markus RP, Sousa KS, da Silveira Cruz-Machado S, Fernandes PA, Ferreira ZS. Possible role of pineal and extra-pineal melatonin in surveillance, immunity, and first-line defense. Int J Mol Sci 2021;22:12143.
16. Takács J, Bódizs R, Ujma PP, Horváth K, Rajna P, Harmat L. Reliability and validity of the Hungarian version of the Pittsburgh Sleep Quality Index (PSQI-HUN): Comparing psychiatric patients with control subjects. Sleep Breath 2016;20:1045-51.
17. Keskin A, Ünalacak M, Bilge U, Yildiz P, Güler S, Selçuk EB, *et al.* Effects of sleep disorders on hemoglobin A1c levels in type 2 diabetic patients. Chin Med J 2015;128:3292-7.
18. Chew M, Tan NY, Lamoureux E, Cheng CY, Wong TY, Sabanayagam C. The associations of objectively measured sleep duration and sleep disturbances with diabetic retinopathy. Diabetes Res Clin Pract 2020;159:107967.
19. Chokroverty S. Overview of sleep and sleep disorders. Indian J Med Res 2010;131:126-40.
20. Zelman DC, Brandenburg NA, Gore M. Sleep impairment in patients with painful diabetic peripheral neuropathy. Clin J Pain 2006;22:681-5.
21. Khalil M, Power N, Graham E, Deschênes SS, Schmitz N. The association between sleep and diabetes outcomes–A systematic review. Diabetes Res Clin Pract 2020;161:108035.
22. Cho EH, Lee H, Ryu OH, Choi MG, Kim SW. Sleep disturbances and glucoregulation in patients with type 2 diabetes. J Korean Med Sci 2014;29:243-7.
23. Shim U, Lee H, Oh JY, Sung YA. Sleep disorder and cardiovascular risk factors among patients with type 2 T2DM. Korean J Intern Med 2011;26:277.
24. Barone MT, Menna-Barreto L. Diabetes and sleep: A complex cause-and-effect relationship. Diabetes Res Clin Pract 2011;91:129-37.
25. Lai YJ, Lin CL, Lin MC, Lee ST, Sung FC, Chang YJ, *et al.* Population-based cohort study on the increase in the risk for type 2 T2DM development from nonapnea sleep disorders. Sleep Med 2013;14:913-8.
26. Hedayati A, Gholampour Y, Dehghan A. The relation between sleep disorders and Hemoglobin A1c levels in patients with type II T2DM. Med J Mashhad Univ Med Sci 2016;59:179-87.
27. Uchmanowicz I, Markiewicz K, Uchmanowicz B, Kołtuniuk A, Rosińczuk J. The relationship between sleep disturbances and quality of life in elderly patients with hypertension. Clin Interv Aging 2019;14:155-65.
28. Hepburn M, Bollu PC, French B, Sahota P. Sleep medicine: Stroke and sleep. Mo Med 2018;115:527-32.
29. Nigam G, Camacho M, Chang ET, Riaz M. Exploring sleep disorders in patients with chronic kidney disease. Nat Sci Sleep 2018;10:35-43.
30. Karthikeyan R, Marimuthu G, Spence DW, Pandi-Perumal SR, BaHammam AS, Brown GM, *et al.* Should we listen to our clock to prevent type 2 T2DM?. Diabetes Res Clin Pract 2014;106:182-90.
31. Bazyar H, Gholinezhad H, Moradi L, Salehi P, Abadi F, Ravanbakhsh M, *et al.* The effects of melatonin supplementation in adjunct with non-surgical periodontal therapy on periodontal status, serum melatonin and inflammatory markers in type 2 T2DM patients with chronic periodontitis: A double-blind, placebo-controlled trial. Inflammopharmacology 2019;27:67-76.
32. Bellon A. Searching for new options for treating insomnia: Are melatonin and ramelteon beneficial? J Psychiatr Pract 2006;12:229-43.
33. Xu J, Wang LL, Dammer EB, Li CB, Xu G, Chen SD, *et al.* Melatonin for sleep disorders and cognition in dementia: A meta-analysis of randomized controlled trials. Am J Alzheimers Dis Other Demen 2015;30:439-47.
34. Johnson AR, Justin Milner J, Makowski L. The inflammation highway: Metabolism accelerates inflammatory traffic in obesity. Immunol Rev 2012;249:218-38.
35. Karamitri A, Jockers R. Melatonin in type 2 T2DM and obesity. Nat Rev Endocrinol 2019;15:105-25.