Title: Tryptophan and Sleep Disruptions in Patients with Celiac Disease

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Abstract

Background: Inflammatory responses in celiac disease (CD) may lead to immune dysregulation and sleep disturbances. Additionally, impaired tryptophan metabolism within the gastrointestinal tract has been identified as being associated with chronic intestinal inflammation. This study examines the link between sleep disorders, tryptophan levels, and cytokine profiles in CD patients.

Methods: A cohort study involving 76 adult CD patients (mean age 40.3 years) was conducted from March to December 2022. Sleep quality was assessed with the Pittsburgh Sleep Quality Index questionnaire. Plasma tryptophan levels were measured using HPLC, and serum *TNF-α* and *IL-10* levels were determined with ELISA. *IL-2* and *IL-4* expression was evaluated using quantitative real-time PCR.

Results: A significant proportion (63.2%) of CD patients experienced poor sleep quality. Additionally, increasing age was positively correlated with the presence of sleep disturbances. Importantly, CD patients with poor sleep quality had lower plasma Trp levels compared to those with good sleep quality ($p<0.0001$). Moreover, individuals with poor sleep quality exhibited elevated *IL-2* ($p=0.03$) level in comparison to patients with good sleep quality. Conversely, there was no significant difference observed in *IL-4*, *IL-10* and *TNF-α* levels between individuals with poor sleep quality and those with good sleep quality.

Conclusions: Low levels of Trp may indicate a potential for Trp supplementation to alleviate sleep disturbances in CD patients. However, further research is needed to understand the underlying mechanisms and evaluate potential interventions.

Keywords: Celiac disease, Sleep quality, PSQI questionnaire, Tryptophan, Immune system

Abbreviations

B2M: Beta-2-Microglobulin

CD: Celiac Disease

DH: Dermatitis Herpetiformis

ELISA: Enzyme-Linked Immunosorbent Assay

GFD: Gluten-Free Diet

5-HT1A: 5-Hydroxytryptamine Receptor 1A

HPA: Hypothalamus-Pituitary-Adrenal

HPLC: High-Performance Liquid Chromatography

IL: Interleukin

NK cell: Natural Killer Cell

PB: Peripheral Blood

PSQI: Pittsburgh Sleep Quality Index

rpm: Revolutions Per Minute

SPSS: Statistical Package for the Social Sciences

RNA: Ribonucleic Acid

Trp: Tryptophan

TNF-α: Tumor Necrosis Factor-alpha

Introduction

Celiac disease (CD) is a chronic autoimmune disorder characterized by inflammatory responses to gluten, a protein found in wheat, barley, and rye (Gujral, Freeman, & Thomson, 2012). CD presents as a complex disorder with a wide range of symptoms, encompassing gastrointestinal manifestations such as abdominal pain and diarrhea, as well as extra-intestinal presentations like dermatitis herpetiformis (DH) and reproductive issues (Therrien, Kelly, & Silvester, 2020). CD patients also commonly report anxiety, depression, and other mood disorders, which may be linked to poor subjective sleep quality (Rostami-Nejad et al., 2020; Sharifnejad et al., 2023; Zingone et al., 2010). Prolonged inadequate sleep is associated with unfavorable outcomes, including the onset of chronic systemic inflammation, which is frequently observed in individuals with CD. (Palumbo & Wyse, 2020; Sobolewska-Włodarczyk et al., 2021).

The intricate interplay between sleep and the immune system highlights how immune activation and inflammation can disrupt sleep patterns. Inadequate sleep can impact immune responses and the production of pro/anti-inflammatory cytokines, such as Tumor Necrosis Factor-alpha (*TNF-α*), Interleukin (*IL*)-*2*, *IL-4*, and *IL-10* (Besedovsky, Lange, & Haack, 2019; Gottshall et al., 2021; Hurtado-Alvarado et al., 2013; M. R. Irwin, 2023; Rockstrom et al., 2018). Additionally, sleep deprivation can elevate cortisol levels by activating the hypothalamus-pituitary-adrenal (HPA) axis, leading to immune activation and the release of inflammatory cytokines like *TNF-α* and *IL-2* (Garbarino, Lanteri, Bragazzi, Magnavita, & Scoditti, 2021; Michael R. Irwin & Opp, 2017). The association between imbalanced inflammatory cytokines and sleep disorders is frequently observed in individuals with CD (Westerholm-Ormio, Garioch, Ketola, & Savilahti, 2002; Zingone et al., 2010).

Tryptophan (Trp), an essential amino acid, is found to be deficient in individuals with CD, as indicated in previous studies (Addolorato et al., 2004; Khalkhal et al., 2022). The primary breakdown of Tryptophan occurs within the Kynurenine pathway, mediated by the enzymes Tryptophan 2,3-Dioxygenase and Indoleamine 2,3-Dioxygenase. This metabolic pathway plays a crucial role in regulating both sleep and immune responses (Fallah et al., 2024; Heimberger & Lukas, 2023). Moreover, Trp serves as a precursor for the production of serotonin and melatonin, both of which are essential for modulating the sleep-wake

cycle (Bhat, Pires, Tan, Babu Chidambaram, & Guillemin, 2020). Recent research also suggests that besides their involvement in sleep regulation, serotonin and melatonin influence immune responses by binding to specific receptors such as the 5-Hydroxytryptamine Receptor 1A (5-HT1A) receptor present on various immune cells, including macrophages and T lymphocytes (Arioz et al., 2019; Herr, Bode, & Duerschmied, 2017).

The relationship between CD and sleep disorders demonstrates a reciprocal nature. CD can negatively impact sleep quality, and inadequate sleep has the potential to worsen the symptoms of CD through immune and inflammatory mechanisms (Ranjbaran et al., 2007). In this study, our primary aim was to evaluate the sleep quality of individuals with CD. To achieve this, we utilized the Pittsburgh Sleep Quality Index (PSQI) questionnaire, a widely recognized tool for assessing subjective sleep quality across various populations. Through the implementation of this questionnaire, we gathered data concerning participants' sleep patterns, duration, problems, and overall sleep quality. Furthermore, we sought to investigate the potential connections between sleep disorder and inflammatory cytokines implicated in CD. Specifically, our focus was on the *TNF-α*, *IL-4*, *IL-10*, and *IL-2*, along with the plasma levels of Trp.

Materials and Methods

Participants

We conducted a prospective study involving a cohort of 76 adult participants from the Celiac Disease and Gluten-Related Disorders Research Center at Shahid Beheshti University of Medical Sciences, Tehran, Iran. The study was conducted between March 20, 2022, and December 28, 2022. Participants in this study were selected based on specific inclusion and exclusion criteria to ensure the integrity of the research. Inclusion criteria included adults aged 18 years and older with a confirmed diagnosis of CD based on positive serological tests and histological biopsies of the small intestine, following the modified Marsh criteria (Rostami et al., 2017). Additionally, participants must not have consumed any tryptophan supplements in the month preceding the study. Individuals were excluded from the study if they had undergone any medical or surgical intervention for CD in the prior three months, had pre-existing sleep

disorders (e.g., sleep apnea, insomnia) unrelated to CD, were currently taking medications that could affect sleep patterns or inflammatory responses (such as corticosteroids), or were pregnant or breastfeeding. Written informed consent was obtained from all participants before their involvement and each participant underwent an aseptic collection of 15 milliliters of venous blood utilizing specialized vacuum tubes.

Sleep Quality Assessment

This study employed the PSQI to evaluate participants' sleep quality. The PSQI questionnaire comprises 19 items classified into seven subscales, including sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep problems, use of sleep medication, and daytime dysfunction. Each component is rated on a scale of 0–3, with a total score ranging from 0 to 21. A cumulative score of ≥6 indicates poor sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989; Farrahi Moghaddam, Nakhaee, Sheibani, Garrusi, & Amirkafi, 2012).

Evaluation of Tryptophan Plasma Levels

To evaluate plasma levels of Trp, the peripheral blood (PB) samples underwent centrifugation at 3500 revolutions per minute (rpm) for 15 minutes. The resulting plasma fraction was then stored at -80°C until needed for analysis by High-Performance Liquid Chromatography (HPLC). The ACME 9000 system, manufactured by Younglin, Anyang, Korea, equipped with a fluorescence detector essential for determining Trp levels, was utilized in this study. To remove proteins from the plasma samples, a solution of methanol was introduced. The deproteinization process involved vortexing the samples for 30 seconds and subsequent centrifugation at 5000 rpm for 7 minutes. The resulting clear supernatant was then prepared for further analysis.

Chromatographic separation of Trp was achieved by injecting 100 µL of the clear supernatant into a GL Sciences column with dimensions of 250 mm × 3.0 mm and a particle size of 3 µm. The mobile phase for this process comprised a mixture of methanol and tetrahydrofuran in a volumetric ratio of 4:1. Throughout

the analysis, the fluorescence signals were monitored and recorded at optimal excitation and emission wavelengths of 340 nm and 450 nm, respectively.

Evaluation of Pro-Inflammatory Cytokines in Serum

The blood samples were allowed to clot for 30 minutes at room temperature before undergoing centrifugation at 1500×g for 10 minutes. Subsequently, serum was collected and stored at -80°C for further biochemical analysis. The concentrations of human *TNF-α* and *IL-10* in the serum were determined using a quantitative sandwich Enzyme-linked immunosorbent assay (ELISA) kit (Karmania Pars Gene, Iran), following the manufacturer's instructions. Absorbance was measured in a microplate reader at 450 nm. Each determination was performed in triplicate, following laboratory principles.

Expression Analysis of Pro-Inflammatory Cytokines

Total ribonucleic acid (RNA) was isolated from whole blood samples of all participants using the YTA Total RNA Purification Mini kit for Blood/Cultured Cell/Tissue (Yekta Tajhiz Azma, Iran) according to manufacturer instructions. RNA concentration and quality were assessed using a Nanodrop 1000 spectrophotometer (Nanodrop Fisher Thermo, Wilmington, DE, USA). After adjusting the RNA concentrations, cDNA synthesis was carried out using the 2 Step 2X RTPCR Premix (Taq) kit (Biofact™, South Korea) and stored at -20°C for quantitative real-time PCR.

The specific primers for amplifying *IL-2*, *IL-4*, and beta-2-microglobulin (*B2M*) as a housekeeping gene were designed using Gene Runner software (version 3.05). The primer sequences were as followed: *IL-2* Forward: 5'-TACATGCCCAAGAAGGCCAC-3', *IL-2* Reverse: 5'-AGCACTTCCTCCAGAGGTTTG-3'; *IL-4* Forward: 5'-CTTTGCTGCCTCCAAGAACAC-3', *IL-4* Reverse: 5'-TTCCTGTCGAGCCGTTTCAG-3'; *B2M* Forward: 5'-CCAGCGTACTCCAAAGATTC-3', *B2M* Reverse: 5'-ATGTCGGATGGATGAAACCC-3'.

The mRNA expression levels of the target genes were evaluated using SYBR Premix Ex Taq (Real Q Plus 2x Master Mix Green-Amplicon, Japan) with the Rotor-Gene® Q real-time PCR system (Qiagen,

Germany). All qPCR reactions were conducted in duplicates, and the mRNA expression level of each gene was calculated following the $2^{-\Delta\Delta Ct}$ Method ($\Delta Ct = \Delta Ct$ target - ΔCt endogenous).

Statistical analysis

Data analysis was conducted utilizing Statistical Package for the Social Sciences (SPSS) version 25.0, developed by SPSS Inc. (Chicago, IL, USA). Graphical representations were generated using GraphPad Prism 8.4.0, a software developed by GraphPad Software, Inc. (San Diego, CA, USA). Descriptive statistics were employed to summarize the characteristics of the study participants, including demographics, clinical features, and sleep quality parameters. An independent samples t-test was conducted to compare two groups of continuous variables with the normal distribution. All findings were reported with a 95% confidence interval of difference. A p-value of < 0.05 was considered significant. The correlation between variables was assessed using Pearson's correlation tests.

Results

Demographic and Clinical Characteristics Of CD Participants

The study primarily involved adult participants, with a mean age of 40.29 ± 11.62 years. A significant majority of the sample, accounting for 77.6%, consisted of females. Fatigue emerged as the predominant symptom among participants, affecting 63.15% of cases.

Comprehensive demographic and clinical data were collected, including information on the duration of CD, adherence to a gluten-free diet (GFD), smoking habits, marital status, and educational attainment. Moreover, a detailed breakdown of the additional symptoms, such as bloating, diarrhea, vomiting, and osteoporosis is presented in Table 1.

Assessment of Sleep Quality in CD Participants

Table 2 provides a thorough analysis of the overall sleep quality and seven specific PSQI subscales among CD patients. Notably, 63.2% of the participants displayed signs of poor sleep quality based on total questionnaire scores (a significant majority (79.16%) of them were women). Concerning subjective sleep quality, 59.2% of participants reported experiencing fairly good quality. Analysis of sleep latency distribution revealed that 30.3% experienced a latency period ranging from 16 to 30 minutes. Regarding sleep duration, the majority (40.8%) reported sleeping for 6 to 7 hours. Habitual sleep efficiency was notably high, with a majority (72.4%) of participants reporting an efficiency of over 85%. Sleep problems was reported by a considerable percentage (61.8%), occurring less than once a week. Furthermore, the majority (78.9%) of participants stated that they did not use any sleep medications. Daytime dysfunction showed variability, with the highest percentage (38.2%) reporting no difficulties at all.

Plasma Trp Concentration in Poor and Good Sleepers

We assessed the plasma concentrations of Trp in individuals diagnosed with CD, categorizing them based on their reported sleep quality as either poor or good. The findings, illustrated in Figure 1, revealed a statistically significant lower plasma Trp concentration among participants who reported poor sleep compared to the group with good sleep quality ($p<0.0001$).

Serum Levels of *TNF-α* and *IL-10* in Poor and Good Sleepers

Serum levels of *TNF-α* and *IL-10* were measured in CD patients with either poor or good sleep quality. No statistically significant difference was observed in the serum levels of *TNF-α* and *IL-10* between the group of individuals experiencing poor sleep and the group reporting good sleep quality ($p>0.05$) (Figure 2).

The Expression Levels of *IL-2* And *IL-4* mRNA in Poor and Good Sleepers

We examined the relative expression of *IL-2* and *IL-4* genes in individuals diagnosed with CD who reported varying levels of sleep quality. As depicted in Figure 3, our findings revealed a significant increase in the

expression of *IL-2* among the group experiencing poor sleep compared to those reporting good sleep (p=0.03). Conversely, the change in the expression level of *IL-4* was not statistically significant (p>0.05).

Correlation Analysis

Pearson's correlation analysis was conducted to assess the relationships between various factors, including age, sex, duration of CD, adherence duration to a GFD, smoking habits, marital status, *IL-2* expression, and plasma Trp levels, with different aspects of sleep quality. The results showed a significant positive correlation between participants' age and sleep problems (p=0.009, r=0.37). Moreover, *IL-2* mRNA levels were negatively correlated with sleep latency (P=0.04, r=-0.57) and daytime dysfunction (P=0.03, r=-0.6). No significant correlations were found between the other variables and different aspects of sleep quality (Table 3).

Primary Awareness Sources of CD Patients

The study delved into the main sources of information regarding CD, dietary considerations, and follow-up for participants. Attendance at medical congresses has been identified as a substantial source of information, representing 52.6% of the sample. This finding highlights the vital importance of professional gatherings in the dissemination of knowledge within the medical community.

Additionally, consultations with physicians were crucial, with 67.1% of participants depending on this source. Most of the participants were observed and followed up by gastroenterologists. The distribution of awareness from other sources is detailed in Table 4.

Discussion

This study represents the investigation into the quality of sleep in Iranian patients with CD. It explores the association between CD and sleep quality, alongside the possible involvement of inflammatory cytokines and Trp in this association. The results reveal that a notable portion (63.2%) of participants experienced poor sleep quality.

Consistent with these findings, a previous study by Zingone et al. (Zingone et al., 2010) also noted high PSQI scores among CD patients, indicating poor sleep quality characterized by prolonged sleep latency and short sleep duration. Ballou et al. (Ballou et al., 2018) similarly observed poor sleep quality in 61% of CD patients, aligning closely with our results. Furthermore, our study identified a significant positive correlation between the age of CD patients and sleep problems, underscoring the importance of integrating sleep evaluations into routine follow-up protocols as patients age. In alignment with this, a study by Marild et al. (Mårild et al., 2015) demonstrated that CD patients are 33% more likely to use hypnotic drugs compared to the healthy control group. In our study, 21.1% of CD patients reported using sleep medications. Importantly, CD patients with poor sleep quality exhibited lower plasma Trp levels compared to those with good sleep quality. Since Trp plays a crucial role in sleep regulation through its involvement in the kynurenine pathway and serotonin synthesis, assessing Trp levels before prescribing hypnotic drugs may offer benefits to CD patients (Bhat et al., 2020; Heimberger & Lukas, 2023). Considering Trp supplementation as a potential primary approach for improving sleep quality in these patients is noteworthy. A study conducted by Sutanto et al. (Sutanto, Loh, & Kim, 2022) demonstrated that the inclusion of Trp effectively reduced sleep latency. Specifically, participants consuming 1 gram or more of Trp exhibited a significantly shorter time to fall asleep compared to those consuming less than 1 gram. However, Trp supplementation did not exhibit significant effects on other sleep aspects.

In our study, individuals with poor sleep quality demonstrated higher levels of *IL-2*, and no changes in *TNF-α*, *IL-4* and *IL-10* compared to those with good sleep quality. These cytokines play a crucial role in immune signaling and have been implicated in both CD pathogenesis and sleep regulation (Imeri & Opp, 2009; Redwine, Hauger, Gillin, & Irwin, 2000). Prior research investigating cytokine levels in individuals with sleep problems have produced varied results. For instance, Taraz et al. found elevated levels of *TNF-α* in the serum of patients undergoing hemodialysis with poor sleep quality, which is consistent with our findings (Taraz et al., 2013). Several studies have demonstrated a correlation between sleep deprivation and elevated levels of *TNF-α* in subsequent days (Kaushal, Ramesh, & Gozal, 2012). The elevated *TNF-α* concentration following sleep deprivation or fragmentation contributes to excessive daytime sleepiness in

patients with sleep apnea (Kaushal et al., 2012). Additionally, Yang et al. demonstrated a positive correlation between poor sleep quality and *TNF-α*, as well as a negative correlation with *IL-2* (Yang et al., 2023). Kaartinen et al. associated good overall sleep quality with higher logarithmic cytokine concentrations of *IL-2*, *IL-4*, *IL-6*, *IL-10*, *IL-12*, and *IL-13* (Kaartinen et al., 2019). In our study, elevated levels of *IL-2* were observed in subjects with poor sleep quality, consistent with previous research indicating that sleep deprivation reduces lymphocyte blastogenesis, natural killer (NK) cell activity, and upregulates *IL-1* and *IL-2* (Ibarra-Coronado et al., 2015). Furthermore, we noted a negative correlation between *IL-2* mRNA levels and sleep latency and daytime dysfunction, emphasizing the importance of this gene in sleep disorders. Research by Kaartinen, M. et al. suggests that good overall sleep quality corresponds to high logarithmic concentrations of *IL-4* and *IL-10* cytokines (Poluektov, 2021). Previous studies have also demonstrated decreased production of stimulated *IL-10* and *IL-4* during sleep in humans, indicating a decline in anti-inflammatory activity during sleep (Poluektov, 2021).

Given the notable female predominance in our cohort (77.6%), it is essential to consider how gender may influence sleep quality and immune responses in patients with CD. Research indicates that women often report poorer sleep quality and are more susceptible to sleep disorders compared to men, which may be attributed to hormonal fluctuations related to the menstrual cycle, pregnancy, or menopause (Nowakowski, Meers, & Heimbach, 2013). Furthermore, the immune response differs between genders, with females generally exhibiting stronger immune reactions, which can influence inflammatory markers like cytokines (Klein & Flanagan, 2016). In our study, the observed elevation of *IL-2* levels among individuals with poor sleep quality underscores a potential pathophysiological link that may be amplified in women due to their distinct immune profile and psychosocial stressors.

Moreover, beside the observed associations between sleep quality, Trp levels, and pro-inflammatory cytokines in CD patients, several confounding factors may also significantly influence sleep outcomes. Psychological stressors, including anxiety and depression, are prevalent among individuals with CD and have been shown to disrupt sleep patterns (Moawad et al.; Staner, 2003). Furthermore, dietary adherence to a GFD can also play a critical role; variations in adherence levels may influence not only inflammatory

responses but also nutritional intake and overall well-being, thus impacting sleep quality (Cotton et al., 2023). Additionally, the presence of comorbid conditions, such as autoimmune disorders or gastrointestinal symptoms, could contribute to sleep disturbances through mechanisms involving chronic inflammation or pain (Khanijow, Prakash, Emsellem, Borum, & Doman, 2015; Zielinski, Systrom, & Rose, 2019). Hence, controlling for these psychological, dietary, and health-related factors in future research is crucial for elucidating the multifaceted relationship between CD, immune dysregulation, and sleep quality.

To further explore the intricate relationship between CD, sleep quality, and immune function, upcoming research should focus on several key areas. Longitudinal studies are essential to assess how sleep quality evolves over time in response to dietary changes or interventions, such as Trp supplementation, and to investigate the long-term effects on inflammatory markers and overall health. Moreover, specific attention should be given to the role of other dietary components and their interactions with sleep quality in CD patients, as dietary adherence can significantly impact both gut health and sleep regulation. Additionally, examining potential differences in sleep quality and inflammatory responses across diverse populations with CD might provide insights into the influence of genetic, cultural, and environmental factors. Such studies could pave the way for tailored therapeutic strategies aimed at enhancing sleep quality and, consequently, the quality of life for individuals living with CD.

This study has several limitations. Firstly, the study sample consisted of only 76 adult participants from a specific research center, potentially restricting the generalizability of the findings to a broader population. Including a larger and more diverse sample would offer a more representative understanding of the relationship between CD, sleep quality, and inflammatory markers. Secondly, the assessment of sleep quality relied on self-reported measures, specifically the PSQI questionnaire. Self-report measures are prone to recall bias and individual interpretation, potentially impacting the accuracy and reliability of the results. Incorporating subjective measures of sleep, such as polysomnography, would yield more robust and precise data on sleep quality.

Our findings reveal that a substantial proportion of CD patients experience poor sleep quality, emphasizing the need to consider sleep assessments as part of routine care for these individuals. Patients with CD may

experience deleterious effects on sleep, resulting in disturbances that can profoundly impact their overall well-being. The observed correlations between age, sleep problems, and cytokine expression underscore the multi-faceted nature of sleep quality and its potential impact on immune and inflammatory responses in individuals with CD. The observed correlation between low plasma Trp levels and impaired sleep quality implies a potential therapeutic avenue through Trp supplementation to alleviate sleep problems in CD patients. In light of these findings, future research should aim to elucidate the underlying mechanisms linking CD, sleep quality, and immune function.

Highlights

63.2% of CD patients reported poor sleep quality, linked to age.

Poor sleep in CD patients correlates with lower plasma tryptophan levels.

Increased *IL-2* levels seen in poor sleepers; no changes in other cytokines.

Plain Language Summary

CD is a chronic autoimmune disorder that causes the immune system to react negatively to gluten, a protein found in wheat, barley, and rye. Many people with CD experience a range of symptoms, including gastrointestinal issues, fatigue, and mood disorders. This study aimed to explore how CD affects sleep quality and to investigate the role of Trp, an important amino acid involved in sleep regulation and immune function.

Conducted from March to December 2022, the study included 76 adults with CD, averaging 40 years old. We used a sleep quality questionnaire and measured Trp levels and certain immune markers in participants' blood. We found that 63.2% of the participants reported poor sleep quality. Older age was linked to worse sleep, and those with poor sleep had significantly lower levels of Trp in their plasma compared to those with good sleep. Conversely, individuals with poor sleep exhibited higher levels of the cytokine *IL-2*, which is involved in inflammation.

These findings suggest that low Trp levels may contribute to sleep problems in CD patients, pointing to the potential benefits of Trp supplementation as a treatment to improve sleep quality. However, more research is needed to fully understand how these factors interact and to explore effective treatment options. The study highlights the importance of regularly assessing sleep in CD patients to improve their overall health and well-being.

Ethical consideration

Compliance with ethical guidelines

Ethical approval for the study was obtained from the Ethical Committee of the Research Institute for Gastroenterology and Liver Diseases (RIGLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.MSP. REC.1397.564), and written informed consent was obtained from all participants before their involvement.

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Authors' contribution

M.R.N: Conceptualization. N.A., B.A., M.M.G., N.T. writing–original draft. M.R.N., S.J.S., H.H., M.R.T. writing–review and editing.

Declaration of interest

The authors declared no conflict of interest

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Figures

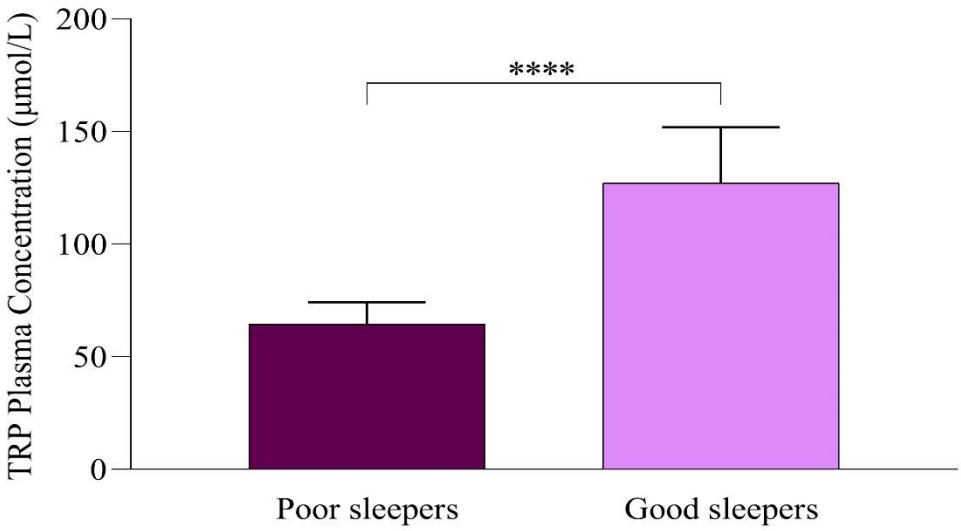

Figure 1.

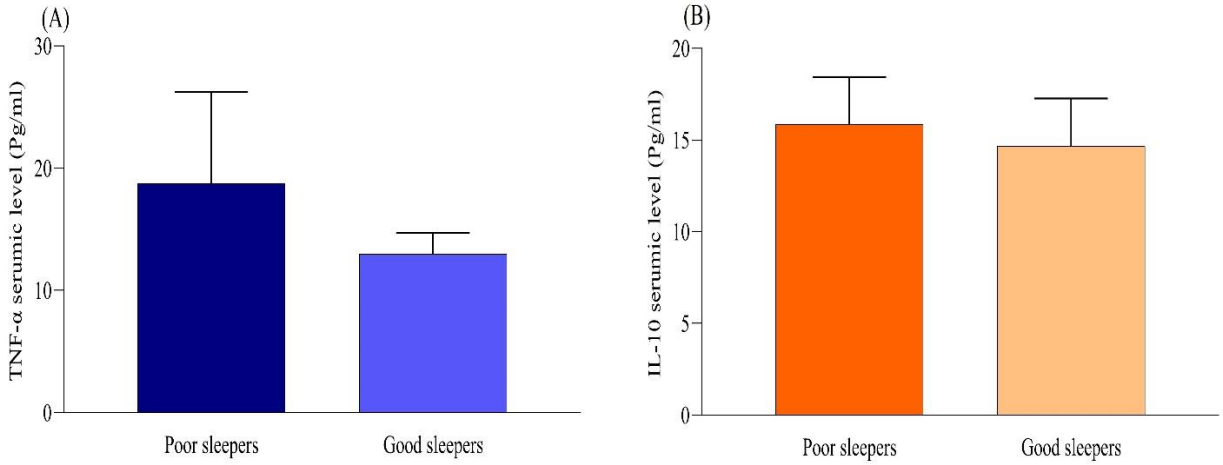

Figure 2.

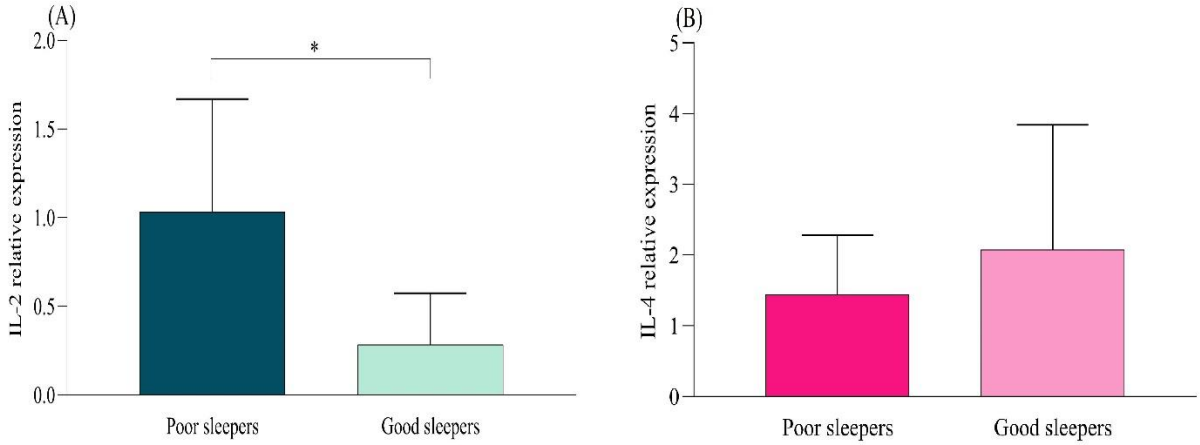

Figure 3.

Tables

Table 1. Demographic and clinical data of studied participants.

Variable		Total sample (n=76)
Age (years)	Mean± SD	40.29±11.62
Age groups	18-30 yr, n (%)	13 (17.10%)
	31-60 yr, n (%)	59 (77.63%)
	More than 61 yr, n (%)	4 (5.27%)
Gender	Males, n (%)	17 (22.4%)
	Females, n (%)	59 (77.6%)
Duration of CD (yr)	Mean± SD	8.13±9.58
GFD adherence duration	≤ 6 months, n (%)	3(3.94%)
	6-12 months, n (%)	3(3.94%)
	≥ 12 months, n (%)	70(92.12%)
Smoking	Yes, n (%)	7(9.21%)
	No, n (%)	69(90.79%)
Marital status	Single, n (%)	29(38.15%)
	Married, n (%)	47(61.85%)
Level of education	≤Diploma, n (%)	43(56.57%)
	BS, n (%)	25(32.89%)
	MS, n (%)	4(5.27%)
	PhD, n (%)	4(5.27%)
Clinical symptoms	Bloating n (%)	46(60.52%)
	Diarrhea n (%)	23(30.26%)
	Fatigue n (%)	48(63.15%)
	Vomiting n (%)	12(15.78%)
	Osteoporosis n (%)	43(56.57%)

Table 2. Global sleep quality and seven Pittsburgh Sleep Quality Index subscales in the total sample of CD patients, in Tehran, Iran, 2022 (n=76).

PSQI	Total sample		
General sleep quality, n (%)			
good	28 (36.8%)	Females, n (%)	21(75%)
		Males, n (%)	7(25%)
poor	48 (63.2%)	Females, n (%)	38(79.16%)
		Males, n (%)	10(20.83%)
PSQI sub-scale			
Subjective sleep quality, n (%)			
Very good	10 (13.2%)		
Fairly good	45 (59.2%)		
Fairly bad	15 (19.7%)		
Very bad	6 (7.9%)		
Sleep latency, n (%)			
< 15 minutes	14 (18.4%)		
16-30 minutes	23 (30.3%)		
31-60 minutes	22 (28.9%)		
> 60 minutes	17 (22.4%)		
Sleep duration, n (%)			
> 7 hours	18(23.7%)		
6-7 hours	31 (40.8%)		
5-6 hours	19 (25.0%)		
< 5 hours	8 (10.5%)		
Habitual sleep efficiency, n (%)			
> 85%	55 (72.4%)		
75-84%	14 (18.4%)		
65-74%	4 (5.3%)		
< 65%	3 (3.9%)		
Sleep problems, n (%)			
Not during past month	6 (7.9%)		
Less than once a week	47 (61.8%)		
Once or twice a week	22 (28.9%)		
Three or more times a week	1 (1.3%)		
Use of sleeping medications, n (%)			
Not during past month	60 (78.9%)		
Less than once a week	4 (5.3%)		
Once or twice a week	3 (3.9%)		
Three or more times a week	9 (11.9%)		
Daytime dysfunction, n (%)			
No problem at all	29 (38.2%)		
Only a very slight problem	22 (28.9%)		
Somewhat of a problem	16 (21.1%)		
A very big problem	9 (11.8%)		

Table 3. Correlation between Sleep Quality Aspects and demographic and clinical status of CD Patients.

Variables		General sleep quality	Subjective sleep quality	Sleep latency	Sleep duration	Habitual sleep efficiency	Sleep disturbance	Use of sleeping medications	Daytime dysfunction
Age	P-value	0.291	0.052	0.962	0.062	0.950	**0.009***	0.094	0.080
	Correlation coefficient	0.12	0.22	-0.005	0.21	0.007	0.37	0.19	-0.2
Sex	P-value	0.831	0.267	0.343	0.573	0.292	0.107	0.840	0.821
	Correlation coefficient	0.02	0.13	0.11	0.06	0.12	0.12	0.2	-0.02
CD duration	P-value	0.351	0.991	0.372	0.105	0.968	0.198	0.187	0.447
	Correlation coefficient	0.11	0.001	0.11	0.2	0.005	0.42	0.16	-0.09
GFD duration	P-value	0.972	0.801	0.850	0.607	0.602	-0.096	0.208	0.636
	Correlation coefficient	0.004	-0.03	-0.02	-0.06	-0.21	-0.09	-0.14	0.05
Smoking	P-value	0.122	0.703	0.932	0.610	0.573	0.760	0.298	0.666
	Correlation coefficient	0.17	-0.04	-0.009	-0.05	0.06	0.03	0.12	0.05
Marital status	P-value	0.091	0.440	0.248	0.968	0.052	0.444	0.766	0.082
	Correlation coefficient	-0.19	0.09	-0.13	0.006	-0.22	0.08	-0.03	-0.19
TRP level	P-value	0.483	0.938	0.540	0.990	0.517	0.135	0.349	0.167
	Correlation coefficient	-0.24	0.02	0.17	0.004	0.2	-0.41	0.22	0.57
IL-2 level	P-value	0.395	0.322	**0.040***	0.630	0.590	0.092	0.262	**0.031***
	Correlation coefficient	-0.26	-0.31	-0.57	-0.16	-0.17	0.76	-0.34	-0.6

Table 4. Database sources of patient's awareness about their disease, diet, and follow-up.

Sources of awareness of:	Frequencies (n, %):
Celiac disease	
Physician	38 (50%)
Social media	34 (44.7%)
Broadcast media	7 (9.2%)
Surfing the net	24 (31.6%)
Congress	40 (52.6%)
Books	17 (22.4%)
Gluten-free diet	
Physician	51 (67.1%)
Social media	15 (19.7%)
Books	7 (9.2%)
Surfing the net	23 (30.3%)
Follow up	
General practitioner	2 (2.6%)
Internist	4 (5.3%)
Gastroenterologist	73 (96.1%)
Traditional medicine	4 (5.3%)

Figure legends

Fig 1 Plasma Trp levels in good and poor sleeper CD patients. Values are presented as means ± SD. CD: Celiac disease, TRP: Tryptophan. **** $p < 0.0001$.

Fig 2 Analysis of (A) *TNF-α* and (B) *IL-10* serum cytokine levels in good and poor sleeper CD patients using ELISA. Values are presented as means ± SD. *IL-10*: Interleukin-10, *TNF-α*: Tumor Necrosis Factor-alpha.

Fig 3 The Mean±SD values of (A) *IL-2* and (B) *IL-4* ($p>0.05$) relative expression levels in Good and Poor sleeper CD patients using real-time PCR assay. Expression of transcripts was normalized to *B2M*. *B2M*: Beta-2-Microglobulin, CD: Celiac disease, *IL-2:* Interleukin-2, *IL-4:* Interleukin-4. * $p=0.03$.