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Acute Sleep Deprivation on Correlates with Biomarkers of Chronic Inflammation in Healthy Individuals

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Abstract

Lack of adequate sleep and irregular sleep patterns stand as independent factors contributing to chronic inflammation. Sleep deprivation also correlates with the onset of various neurodegenerative diseases, marked by brain protein accumulation leading to memory loss and cognitive decline. This study aims to investigate the connection between sleep deprivation and the pro-inflammatory markers aiming to enhance our comprehension of this relationship and potentially identify future intervention possibilities. The study was conducted with 50 individuals as participants of which 25 were sleep-deprived and 25 had adequate amounts of sleep. The sleep duration details of the individuals were obtained by questionnaire. Blood was withdrawn from all the subjects after due consent from them. Plasma levels of S-100, acetylcholinesterase (AchE), C-reactive protein (CRP) and Complement 3 (C3) were assessed using the ELISA method in the fasted state. The results demonstrated a significant positive correlation between acute sleep deprivation and increased levels of S-100, AchE, CRP and C3 levels in the sleep-deprived individuals when compared to individuals who had adequate sleep. Our exploratory study results suggest that sleep deprivation is associated with increased levels of pro-inflammatory markers in the body. These changes reinforce the notion that sleep deprivation may have detrimental effects on brain health, even in younger individuals. It's essential to study larger groups to distinguish between the impacts of sleep loss and circadian rhythms, understand implications for persistent conditions like those in shift workers, and explore how these effects might interact with other lifestyle choices and genetic factors.

Keywords: *Biomedical Research, Biomarkers Sleep, Health, Inflammation, Sleep Deprivation.*

Introduction

While the significance of sleep for survival and cognitive performance has been acknowledged and researched for more than a century, it's only within the past decade that scientific investigations have commenced to systematically reveal the reciprocal connection between sleep and the well-being of the central nervous system (CNS) [1-3]. Variability in sleep, termed intraindividual variability or night-to-night variability in sleep patterns, has gained significance as a crucial measure for understanding sleep and its impact on physical and mental well-being [4, 5]. Traditionally, inadequate sleep is characterized by increased time spent awake, reduced time spent asleep, and decreased sleep efficiency [6]. Earlier studies propose that, in addition to average

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sleep duration, irregular sleep patterns serve as significant markers of sleep disturbances [7, 8]. Emerging evidence suggests that disrupted sleep plays a role in cognitive decline and may heighten the risk of various pathophysiological mechanisms within the brain [9-11]. The reciprocal connection between sleep patterns and neurodegeneration is substantiated by the progress in comprehending how sleep disruptions elevate systemic inflammation, considered an early event in the progression of neuronal damage [12]. Insufficient sleep has been linked to elevated levels of inflammatory markers [6-8]. Though the exact mechanism by which altered sleep affects health is unknown, endeavours have aimed to identify molecular biomarkers, including altered gene expression or metabolite profiles influenced by sleep deprivation.

Inflammatory cytokines are known to regulate and be regulated by sleep and include a wide variety of regulatory proteins such as chemokine, interferons, interleukins, tumour necrosis factor, etc [13]. The level of pro-inflammatory cytokines is known to be elevated in sleep deprivation and this is believed to be a major contributor to the development and progression of various chronic inflammatory diseases such as diabetes, atherosclerosis, neurodegenerative disorders, etc [14, 15]. Some experimental research based on sleep deprivation has shown that sleep deprivation has acutely elevated inflammatory cytokine levels [16, 17]. Sleep deprivation triggers a pathogen-independent mechanism that heightens low-grade inflammation and activates microglia, resulting in impaired cellular immunity, elevated levels of pro-inflammatory agents like TNF-α, IL-6, complement system proteins and C-reactive protein (CRP), and changes in vascular function [12, 17].

The S100 protein, a calcium-binding protein, is implicated in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Within the central nervous system, oligodendrocytes, astrocytes, and neurons express S100 proteins under both normal and pathological conditions. While amyloid-beta aggregation and hyperphosphorylated tau plaques represent the primary pathological indicators in Alzheimer's disease, the S100 protein family is strongly linked to neuroinflammatory processes across various neurodegenerative conditions [18]. Also, acetylcholine (Ach) has been shown to possess a strong association with memory functions and neurodegeneration. Plasma levels of Ach was found to be depleted in patients with cerebral infarction and insomnia [19]. Therefore, assessing the levels of these markers in plasma samples will scores serve as indicators of heightened allostatic load, irrespective of any underlying chronic health conditions.

Further, complement C3 is an important protein in the complement system and is also known to play an important role in the activation of the complement system and immune or inflammatory responses during which their levels are up-regulated. Both complement c3 and c4 levels are known to be increased in chronic inflammatory conditions as well as in sleep deprivation conditions [20-22]. C-reactive protein or CRP is an acute phase reactant protein is an acute- phase reactant protein which is synthesized by the liver. Through various studies, it has been established that CRP levels rise during inflammation and hence are being used as a reliable marker for inflammation in the diagnosis of various medical conditions [23, 24]. In line with this established evidence associating insufficient sleep with increased risk in specific bodily systems, our hypothesis posited that individuals experiencing shorter sleep durations would exhibit elevated multisystem biological risk scores. Therefore, the purpose of this study is to find the relation between sleep deprivation and levels of pro-inflammatory markers S-100, AchE, CRP and complement C3. Since only a limited number of studies have been performed on this particular topic this research may help

us gain more knowledge about the topic and be used for future interventions.

Methodology

Ethics Approval/ Consent to Participate

This study was approved by the IHEC Committee, Saveetha Medical College & Hospitals and all the participants who contributed to this study declared their consent to participate.

Participants

The study was conducted with 50 healthy individuals as participants, of which 25 had sleep disturbances and 25 had an adequate amount of sleep. The sleep duration details of the individuals were obtained using a questionnaire who visited the Sleep Laboratory, Saveetha Hospitals with complaints of disturbed sleep, fragmented sleep, and irregular sleep patterns with an overall sleep duration of <5h/day were selected for the study and normal healthy individuals who had adequate sleep time of 7-8h/day were selected for the control group.

Inclusion Criteria

For participation, healthy individuals of any gender within the age range of 18-35 were considered. Individuals who were confirmed to have been sleep deprived and/or having irregular sleep patterns with sleep duration <5h/day by the departments were selected for the study and normal healthy individuals were selected for control.

Exclusion Criteria

Participants with uncontrolled systemic illnesses such as diabetes, congestive heart failure, renal or hepatic disease, a history of anxiety or depression, or those taking medications other than non-sedating antihistamines or oral contraceptives were excluded from the pool of participants. Nightshift workers and individuals with smoking and alcohol habits were also excluded from the study.

Sample Collection

Patients who satisfied the inclusion and exclusion criteria were selected for the study. Blood samples from the 50 individuals were collected after obtaining approval from the IHEC committee of Saveetha Medical College and Hospitals. The blood samples were centrifuged at 1,500 rpm and the plasma samples were collected and stored at -80^{o}C for ELISA analysis.

Measures

Pro-inflammatory markers S100, AchE, CRP and complement C3 were used as markers of inflammation for this study. Sandwich-type ELISA Analysis was performed on the samples obtained from the patients and the levels of the above-mentioned markers were measured in both sleep-deprived individuals and healthy controls.

Statistical Analysis

The results from the sandwich ELISA procedure were presented as Mean±SD. Statistical comparisons were performed using a paired t-test, non-parametric Mann-Whitney U test, and Kruskal-Wallis X^2. A significance level of $p<0.05$ was considered statistically significant. The analysis was conducted using Graph Pad Prism software version 8.0.

Results

Estimation of S-100B Levels

The pairwise t-test comparison demonstrated that the plasma concentration of S-100B evaluated in sleep deprivation was found to be significantly ($p<0.05$) increased as compared with that of the healthy individuals who had sufficient sleep (Figure 1).

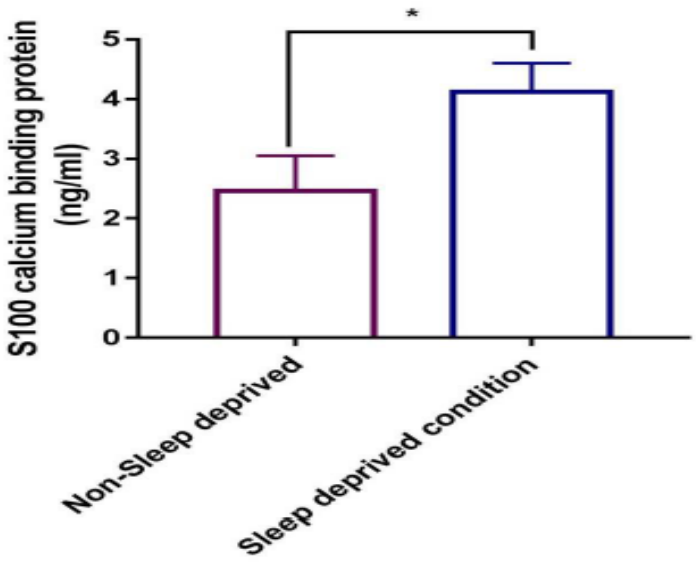

Figure 1. Bar Graph Showing Plasma Concentrations of S-100b in Non-Sleep Deprived and Sleep Deprived Individuals

Normally Distributed Continuous Variables were Described by Mean± SEM and Student T-tests were performed.

*p<0.05, **p<0.01, ***p<0.001 vs Control.

Estimation of Acetylcholinesterase (AchE) Levels

The results of the ELISA analysis carried out to estimate AchE exhibited a marked elevation in the levels of AchE in sleep sleep-deprived group whereas the non-sleep-deprived individuals exhibited no elevation (Figure 2).

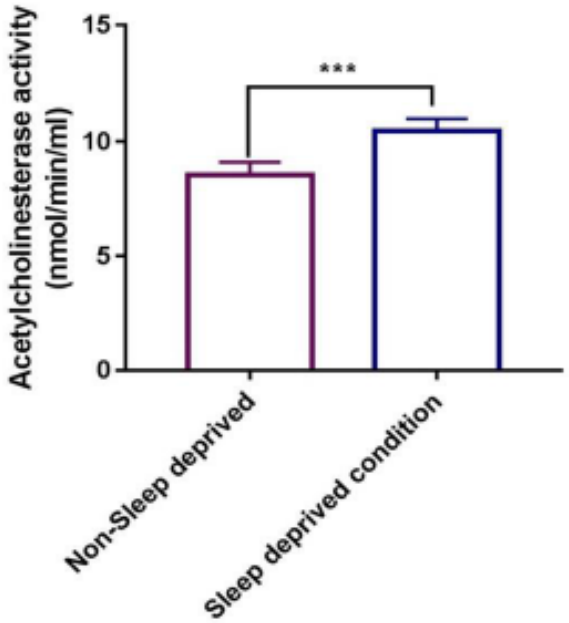

Figure 2. Bar Graph Showing Plasma Concentrations of Ache in Non-Sleep Deprived and Sleep Deprived Individuals

Normally Distributed Continuous Variables were Described by Mean± SEM and Student T-tests were performed.

*p<0.05, **p<0.01, ***p<0.001 vs Control.

Estimation of C-Reactive Protein Levels

The bar graph shows significantly elevated levels ($p<0.001$ by student's t-test) of CRP in the sleep-deprived individuals than that of individuals who had adequate sleep (Figure 3).

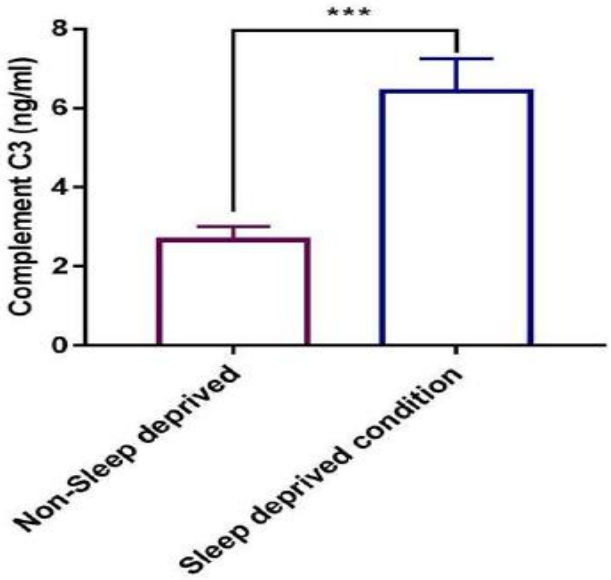

Figure 3. Bar Graph Showing the Levels of CRP in Non-Sleep Deprived and Sleep Deprived Individuals

Normally Distributed Continuous Variables were Described by Mean± SEM and a Student T-Test was performed.

*p<0.05, **p<0.01, ***p<0.001 vs Control.

Estimation of Complement C3 Levels

Complement components play a major role in the activation of inflammatory pathways. In the present study, we assessed the levels of C3 in SD conditions. The results demonstrated a significant ($p<0.001$) increase in the concentration of C3 in comparison to that of subjects who had sufficient sleep (Figure 4).

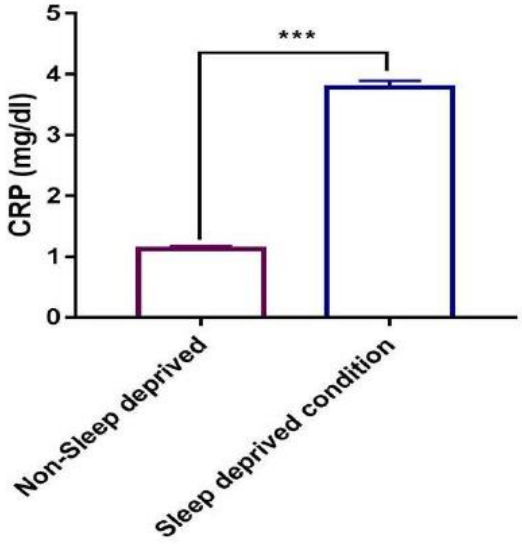

Figure 4. Bar Graph Showing the Levels of Complement C3 in Non-Sleep Deprived and Sleep Deprived Individuals

Normally Distributed Continuous Variables were Described by Mean± SEM And the Student's T- Test was Performed.

*p<0.05, **p<0.01, ***p<0.001 vs Control.

Discussion

The present study aimed to explore the connection between lack of sleep and persistent inflammation, an early factor in neurodegenerative diseases. This research introduced a novel investigation into atypical cytokine biomarkers potentially associated with sleep disruptions and/or the advancement of neurodegeneration. Our findings revealed that individuals experiencing ≤5 hours of sleep exhibited significantly elevated levels of S-100B, AchE, CRP, and complement C3 compared to those with adequate sleep.

Earlier studies investigating sleep deprivation consistently reveal a swift elevation in pro-inflammatory cytokines, a protein group governing both sleep and inflammation [6, 12]. Robust connections have been established between cognitive assessments and plasma biomarkers (CRP, TNF-α, and IL-10), with significant associations persisting even after adjusting for covariates. Presently, limited literature directly contrasts specific cognitive measures with levels of these inflammatory plasma biomarkers, restricting direct comparisons to our study. Nevertheless, previous research indicates that inflammatory biomarkers correlate with diverse cognitive changes, such as changes in recall patterns and cognitive decline [25-27]. CRP, an acute-phase protein originating from the liver, stands as a dependable marker for diagnosing various inflammation-associated medical conditions [7, 16]. Sleep deprivation exhibits notable impacts on CRP, interleukins, and tumour necrosis factor-alpha (TNF-alpha) [6]. These markers hold ties to chronic inflammation and an escalated risk of developing inflammatory diseases like cardiovascular disease, diabetes, and neurodegenerative disorders [9, 28, 29].

Complement C3 stands as a pivotal protein within the complement system, crucial for immune activation and instigating inflammatory responses, marked by heightened levels during these processes [20-22]. A prior investigation involving a 24-hour sleep deprivation period among ten healthy volunteers (comprising 5 males and 5 females) exhibited a significant surge in immunoglobulins such as IgM, IgA, and IgG, alongside complement proteins C3 and C4 [30]. A preclinical rat model study similarly displayed escalated levels of C3, C3a, C5, and C5a proteins in the hippocampal region following sleep deprivation [31]. Building upon prior discoveries, our current study also unveils augmented protein levels of CRP and C3, suggesting the onset of inflammation and compromised immune function in sleep-deprived individuals. However, contradicting these observations, an alternate study has presented evidence showcasing the prevention of complement system activation amid sleep deprivation, evident in reduced levels of C3 and C4 [32].

Sleep deprivation has been linked to the impairment of hippocampal cholinergic system function, potentially leading to memory deficits [19]. Studies in animals have demonstrated lowered Ach levels during sleep deprivation, suggesting increased AchE activity [33-35]. A recent investigation highlighted alterations in synaptic ultrastructure, including changes in mitochondria, basal lamina, synaptic proteins, synaptic vesicles, and junctional folds crucial for neuromuscular transmission during acute sleep deprivation. Additionally, acute sleep deprivation revealed reduced acetylcholine levels and heightened activity of acetylcholine esterase at neuromuscular junctions [36]. Clinical data align with these findings, showing compromised plasma Ach levels in patients with insomnia and cerebral infarction [19]. Our study's results are consistent with prior research, indicating elevated acetylcholinesterase levels, implying a reduction in Ach levels. Given acetylcholine's critical role in memory functions, our findings suggest that individuals experiencing sleep deprivation with heightened AchE levels may face future memory impairments.

Further, in the present study, we observed increased protein levels of S-100B in sleep-deprived individuals which is by a previous study that demonstrated elevated S-100B after a single night of sleep deprivation [37]. The oxidation of substrates culminates in the generation of reactive oxygen species (ROS), including hydrogen peroxide. Studies prior have shown that reactive oxygen species (ROS) possess the capability to harm neurons and potentially trigger cell death. Therefore, it may be speculated that the elevation in S-100B levels in sleep-deprived individuals is due to increased ROS due to sleep deprivation [38, 39]. Moreover, studies have exhibited a close association between increased S-100 levels with concomitant increase in amyloid beta formation and tau phosphorylation which is the hallmark of Alzheimer's disease [40-42].

Conclusion

In conclusion, the results of the present study exhibited the involvement of inflammatory signalling pathways in sleep deprivation conditions. Further, the present study on sleep deprivation involved individuals who typically have normal sleep patterns but experienced short-term sleep deprivation. Therefore, the conclusions drawn from the present study may not be relevant or applicable to populations of people who regularly experience insufficient sleep or have a habitual pattern of short sleep duration. To gain a more comprehensive understanding of the effects of sleep deprivation on different groups, future research should include participants with diverse sleep patterns and durations. This approach would enable researchers to better grasp how sleep deprivation impacts various populations and design more tailored interventions accordingly.

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Conflict of Interest

The authors declare no conflict of interest.

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