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Polysomnographic correlates of inflammatory complement components in young healthy males



M. Ejaz Hussain^a, Abu Hasnath M. Golam Sarwar^b, Mohd. Shoeb Alam^c,
Majumi M. Noohu^a, Wassilatul Zannat^a, Seithikurippu R. Pandi-Perumal^d,
Ahmed S. Bahammam^{e,f}, Md. Dilshad Manzar^{a,*}

^a Centre for Physiotherapy and Rehabilitation Sciences, Jamia Millia Islamia, New Delhi, India^b Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, India^c Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India^d Somnogen Canada Inc., Toronto, ON, Canada^e The University Sleep Disorders Centre, Department of Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia^f The Strategic Technologies Program of the National Plan for Sciences and Technology and Innovation in the Kingdom of Saudi Arabia, Saudi Arabia

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ABSTRACT

A growing body of evidence has delineated the predominant role of humoral mediators of inflammation in linking sleep with immunity. Nonetheless, characterization of the relationship between complement components with inflammatory functions and objective sleep measures has not been performed. In this study we investigated the relationships between objective measures of sleep and complement components with inflammatory functions. Thirty-six healthy male university students (age, 23.94 ± 4.23 years; BMI, 23.44 ± 2.67 kg/m²) completed the study. An RMS Quest 32 polysomnograph (PSG) was used for sleep recording. Non-fasting blood was collected before subjects went to bed on the second night in the sleep laboratory to estimate complement component 3 (C-3), complement component 4 (C-4), complement factor-H (Factor-H), C1-inhibitor (C1INH), complement factor I (CFI) and other inflammatory mediators, such as IL-6 and sICAM-1. Multiple linear regression analysis was used to assess the association between PSG sleep measures and inflammatory mediators. Higher values of C-3 and lower values of sICAM-1, C1INH, and CFI (adjusted model, $R^2=0.211$, $p < 0.041$) predicted longer sleep duration. Lower C-3 (adjusted model, $R^2=0.078$, $p < 0.055$) predicted higher N1 (%). Higher levels of C1INH and CFI and lower values of C-4 (model adjusted $R^2=0.269$, $p < 0.008$) predicted higher N3 (%). Higher C-3, higher C-4, lower IL-6, lower C1INH and lower CFI (model adjusted $R^2=0.296$, $p < 0.007$) predicted higher REM (%). Poor sleep measures were associated with increased levels of pro-inflammatory complement components and decreased anti-inflammatory complement components.

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1. Introduction

Complement is a complex innate immune surveillance system that plays a pivotal role in defense against pathogens and in host homeostasis [1,2]. This complement system assists antibodies and phagocytes in removing pathogens from the organism. The system

is composed of approximately 30 molecules, some of which play an important role in the inflammation mechanism [3,4]. Complement components mediate inflammation upon activation as a consequence of the imbalance in the crosstalk between various serum mediators of this protective mechanism [5,6]. The intricate balance among the inflammatory mediators in the serum is disrupted during sleep problems [7].

Slow wave sleep (SWS) has several important functions, which include roles in cerebral restoration and recuperation in humans [8,9]. Moreover, SWS contributes to the recovery process, up-regulation of the production of pro-inflammatory cytokines, generation of a pro-inflammatory hormonal milieu and suppression of anti-inflammatory hormones [10]. A growing body of data has demonstrated a link between sleep and inflammatory mediators [6,7,12]. Few studies have explored the serum pattern of complement components from the perspective of sleep with varying

* Corresponding author: Present address at Department of Biomedical Sciences, College of Health Sciences, Mizan Tepi University (Mizan Campus), Ethiopia.

E-mail addresses: ehusain@jmi.ac.in (M.E. Hussain),

abuhasnath@gmail.com (A.H.M. Golam Sarwar),

shoebalam_biotech@yahoo.co.in (Mohd.S. Alam),

majuminoohu@gmail.com (M.M. Noohu), zannatw@gmail.com (W. Zannat),

pandiperumal2015@gmail.com (S.R. Pandi-Perumal),

ashammam2@gmail.com (A.S. Bahammam),

md.dilshadmanzar@gmail.com (Md.D. Manzar).

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results. However, these studies have certain limitations, such as the use of heterogeneous sleep loss protocols and screening for limited numbers of complement components [13–15]. We have recently reported the relationship between subjective sleep quality measures and inflammatory complement components [16]. However, the generalizability of those findings to normal objective polysomnographic (PSG) measures of sleep is difficult to appraise [6,13,14,16]. Furthermore, for a better assessment of the association between sleep and inflammatory mediators, objective measurement of sleep parameters is recommended [17].

We hypothesized that the higher level of pro-inflammatory complement components and/or lower level of anti-inflammatory complement components predict poor PSG sleep measures. Therefore, we conducted this study to assess the association between the serum levels of the complement components and PSG sleep measures. Additionally, the serum levels of IL-6 and sICAM-1 were estimated, to aid in the one-point comparative assessment of the associative dynamics of the inflammatory complements vis-à-vis other classes of inflammatory molecules.

2. Materials and methods

2.1. Ethical clearance, participants, and study design

In this cross-sectional, observational study, volunteers were selected after clinical interview for exclusion and inclusion criteria. The advertisement for volunteers was put on university website, and notice boards of the departments, centers, faculty and hostel offices of the varsity. Inclusion criteria included healthy male university students. None of the participants reported treatment for non-communicable chronic conditions, e.g., sleep disorders, neurologic disorders, diabetes, cardiovascular diseases, and psychological/psychiatric (depression, stress) illnesses. None of the participants reported major injury, chronic pain conditions, surgery or use of narcotics. The participant characteristics are given in Table 1. The study sample consisted of 36 unmarried, male, university students. The volunteers were enrolled in various undergraduate ($n=16$) and postgraduate ($n=20$) courses at the university. The sample comprised young ($\text{age}=23.94 \pm 4.23$ yr) university students of north Indian and south Indian (Malayali) ethnicities.

A complete explanation regarding the purpose and procedures of the study was given to the participants. Written informed consent was obtained from all participants prior to enrollment, in compliance with the institutional ethics guidelines and the

Helsinki Declaration. The study was approved by the Human Institutional Ethics Committee, Jamia Millia Islamia, New Delhi, India.

2.2. Sleep evaluation: PSG

The overnight PSG sleep recording was performed with an RMS Quest 32 polysomnograph (Recorders and Medicare Systems, Chandigarh, India). The participants underwent two PSGs. The first night served as an adaptation night to the sleep laboratory conditions (data from this night were not included in the analysis). The PSG records were scored in accordance with the AASM manual, 2007 [18] by a well-trained experienced sleep scorer. The standard recommendations for EEG (three derivations; F4-M1, C4-M1 and O2-M1), EOG (two derivations; E1-M1 and E2-M2), chin EMG, ECG (two electrode), limb movement (piezocrystal electrode), and blood oxygen saturation level (SPO2) were employed.

2.3. Blood extraction, serum separation, storage and ELISA

Blood was taken from the anti-cubital vein 1–2 h before the subjects' bedtime on the second night in the sleep laboratory. Blood was kept undisturbed at room temperature for 30 min for clotting and was then centrifuged at 5000 rpm for 5 min at 4 °C for serum separation. The isolated serum samples were kept frozen at –80 °C prior to quantification. The protocol was in consideration of the published reports of the secondary structure stability, complement integrity and bactericidal activity maintenance [19,20].

Serum C-3, C-4, sICAM-1, C1 inhibitor (C1INH), complement factor-H (Factor-H), complement factor I (CFI) and IL-6 were assayed using commercially available ELISA kits: sICAM-1 and IL-6 (Thermo Fisher Scientific Inc., Rockford, USA); Factor-H, C-4 and C-3 (Abcam, Cambridge, UK); CFI and C1INH (Santa Cruz Biotechnology, Inc. New Bond St Bath, UK). The ELISA testing was performed according to the manufacturers' protocols.

2.4. Statistical analysis

Descriptive statistics were used to assess the mean \pm standard deviation of the normally distributed parametric variables, and the median \pm inter-quartile range was used for non-normally distributed parametric data and/or non-parametric variables. Multiple linear regression analysis was used to assess the relationship between sleep and inflammatory mediators. Analysis was performed separately for each of the selected PSG sleep measures with a joint multivariate outcome defined by all seven inflammatory mediators. Inclusion of variables was dependent on earlier reports and theoretical considerations. Nine PSG sleep measures, i.e., TST (min), sleep onset latency (SOL), REMOL, WASO, awake time (%), N1 (%), N2 (%), N3 (%) and REM (%), were chosen for assessment of the association with inflammatory mediators. These measures represent different domains of sleep, and prior studies have indicated a possibility of their association with inflammatory mediators [6,12,21]. Two-sided tests were used for all statistics, and the level of significance was set at $p < 0.05$. Some results with $p < 0.01$ are also reported to show the trends. Variables with non-Gaussian distributions were transformed to logarithmic and square root derivations for regression analysis. SPSS 16.0 (SPSS Inc., Chicago, Illinois) was used for the statistical analysis.

Table 1
Participant's characteristics.

Characteristics	Mean \pm SD/frequency
Age (yr)	23.94 \pm 4.23
Height (cm)	167.22 \pm 4.27
Weight (kg)	65.50 \pm 7.38
BMI (kg/m ²)	23.44 \pm 2.67
Under-weights	01
Normal	25
Over-weight	10
Ethnicity	
South Indian (Malayalee)	20
North Indian	16
Courses	
Under-graduate	16
Post-graduate	20
Alcohol consumer	01
Smokers	04

BMI: body mass index.

Table 2
Sleep measures and inflammatory mediators.

Sleep measures/Inflammatory mediators	Mean \pm SD/Median (IQR)
TST (min)	374.02 \pm 65.98
SOL (min)	8.50 (7.38) ^a
SE (%)	93.59 \pm 3.55
REMOL (min)	92.00 (90.38) ^a
WASO (min)	6.25 (20.38) ^a
Awake time (%)	5.17 (6.10) ^a
N1 (%)	6.00 (6.21) ^a
N2 (%)	43.36 \pm 8.25
N3 (%)	26.38 \pm 9.77
REM (%)	17.16 \pm 5.21
ODI	4.33 \pm 2.93
Mean SpO ₂	95.90 \pm 0.83
C3 (μ g/ml)	1442.00 \pm 109.48
C4 (μ g/ml)	453.58 \pm 44.21
Factor-H (μ g/ml)	515.28 \pm 127.84
C1INH (μ g/ml)	193.14 \pm 27.37
CFI (μ g/ml)	64.55 \pm 5.37
IL-6 (pg/ml)	1.32 \pm 0.09
sICAM-1 (μ g/ml)	0.19 \pm 0.03

TST: Total sleep time, SOL: sleep onset latency, SE: sleep efficiency, REMOL: REM onset latency, WASO: Duration of wake after sleep onset, NREM: Non-rapid eye movement sleep, N1: sleep stage N1, N2: sleep stage N2 and N3: sleep stage N3, REM: Rapid eye movement sleep, ODI: oxygen desaturation index, Mean SpO₂: mean oxygen saturation.

C3: Complement Component-3, C4: Complement Component-4, Factor-H: Complement Factor-H, C1INH: C1-inhibitor, CFI: Complement Factor I and IL-6: Interleukin-6, sICAM-1: soluble Intercellular Adhesion Molecule-1.

^a Median (IQR).

3. Results

3.1. Sleep measures and inflammatory mediators

The descriptive statistics of the PSG sleep measures and inflammatory mediators are shown in Table 2. Six of the sleep measures, i.e., SOL, REMOL, WASO, awake time (%), and N1 (%), were not normally distributed; therefore, their values are shown as the median (inter-quartile range) (Table 2). The PSG showed that the participants had no sleep disorders. The serum levels of all measured inflammatory mediators were within the normal range.

3.2. Association between sleep measures and IIPs

Multiple linear regression analyses were performed individually for each PSG sleep measure to determine the extent to which the inflammatory mediators predict changes in sleep parameters. BMI and BMI² were entered in one block along with the 7 inflammatory mediators as independent variables, and the backward method was used. The multiple regression analysis

results of 6 of the 9 PSG sleep measures are presented in Table 3. SOL, WASO, and awake time (%) were not associated with inflammatory mediators. REMOL and N1 (%) were log-transformed to satisfy the normality distribution requirement of the regression analysis.

Longer TST was predicted by higher C-3, lower sICAM-1, lower C1INH, lower CFI, higher BMI and lower BMI² (model adjusted R²=0.211, $p < 0.041$). Longer REMOL was predicted by lower serum C-3 level and higher BMI (model adjusted R²=0.291, $p < 0.001$). Higher N1 (%) was predicted by lower C-3 (model adjusted R²=0.078, $p < 0.055$). Greater N2 (%) was predicted by a lower serum level of sICAM-1, lower C1INH, higher serum Factor-H and higher BMI (model adjusted R²=0.308, $p < 0.004$). Greater N3 (%) was predicted by a higher serum level of C1INH and CFI, lower BMI² and lower serum C-4 (model adjusted R²=0.269, $p < 0.008$). A higher REM % was predicted by higher C-3, higher C-4, lower IL-6, lower C1INH and lower CFI (model adjusted R²=0.296, $p < 0.007$).

4. Discussion

The results support our hypothesis that the pro/anti-inflammatory complement components can predict PSG sleep measures. The associations between objective sleep measures and inflammatory complement components are similar to those between objective sleep measures and inflammatory cytokines. The majority of the selected PSG sleep measures (66.67%) were predicted by inflammatory complement components and other inflammatory mediators. These results reaffirm the conclusions of previous studies of the existence of an association between objective sleep measures and inflammatory mediators [21–24]. Our results concur with a previous study in healthy individuals that has shown that the inflammatory molecules IL-6 and sICAM-1 are not associated with SOL [21]. Our results extended this study, showing that inflammatory cytokine IL-6, inflammatory cell adhesion molecule sICAM-1 and inflammatory complement components were not associated with SOL (Table 3). Lower sleep duration (TST) was associated with higher sICAM-1, C1INH, CFI and lower C-3. C-3 and sICAM-1 have pro-inflammatory functions [25,26]. C1INH, Factor-H and CFI are anti-inflammatory in nature [3,27]. Lower sleep duration is associated with increased serum inflammatory marker TNF- α [23]. Patel et al. have found that reduced PSG sleep duration is associated with increased levels of pro-inflammatory cytokine TNF- α . The authors concluded that extremes of sleep duration are associated with inflammation [23]. Moreover, similarly to earlier reports [28–30], in this male-exclusive study, no association between sleep duration and IL-6 was found. In contrast to earlier reports, we did not find an association

Table 3
Multiple regression predictors of polysomnographic sleep measures.

Sleep variable	Significant individual predictor variables (β coefficients; P values)	Model unadjusted R ² ; adjusted R ² ; P value
TST	C-3 (0.941, 0.001); sICAM-1 (–0.337, 0.069); C1INH (–0.733, 0.002); CFI (–0.638, 0.011); BMI (4.997, 0.066); BMI ² (–4.709, 0.080)	0.346, 0.211, 0.041
REMOL ^a	C-3 (–0.351, 0.020); BMI (0.494, 0.002)	0.332, 0.291, 0.001
N1 (%) ^a	C-3 (–0.323, 0.055)	0.104, 0.078, 0.055
N2 (%)	sICAM-1 (–0.275, 0.076); Factor-H (0.565, 0.003); C1INH (–0.383, 0.033); BMI (0.573, 0.001)	0.387, 0.308, 0.004
N3 (%)	C-4 (–0.653, 0.015); C1INH (0.487, 0.010); CFI (0.357, 0.031); BMI ² (–0.453, 0.009)	0.353, 0.269, 0.008
REM (%)	C-3 (0.482, 0.041); C-4 (0.588, 0.046); IL-6 (–0.463, 0.014); C1INH (–0.409, 0.054); CFI (–0.797, 0.004)	0.397, 0.296, 0.007

TST: total sleep time, REMOL: REM onset latency, WASO: duration of wake after sleep onset, NREM: non-rapid eye movement sleep, NREM1, NREM2, NREM3: represent sub-stages 1, 2 and of NREM, REM: rapid eye movement sleep.

C-3: Complement Component 3, C-4: Complement Component 4, sICAM-1: Intercellular Adhesion Molecule 1, Factor-H: complement factor-H, C1INH: C1-inhibitor, CFI: Complement factor I.

^a Logarithmic transformed for making distribution normal.

between IL-6 and REMOL (Table 3) [21,22]. The samples in previous studies included hypertensive individuals, which may have confounded the results because hypertension is associated with IL-6 [31]. Similarly to earlier reports, our study did not find an association between N1 (%) and IL-6 [21,22]. However, N1 (%) was negatively associated with anti-inflammatory complement component Factor-H (Table 3).

Both C-3 and C-4 were significantly associated with REM sleep (Table 3). This result is striking, given that there is commonality between the rhythm pattern of C-3, C-4, and REM sleep regulation. REM sleep has a predominant circadian regulation [32], and the serum levels of both C-3 and C-4 have well-characterized circadian patterns [14], which gives further insight into the possible physiological basis of our experimental hypothesis, i.e., the similarity between the association pattern of sleep and inflammatory cytokines and the association pattern of sleep and inflammatory complement components. The circadian aspect of sleep maintains stable and healthy cytokine rhythms [33] and may play a role in the maintenance of stable and healthy complement component rhythms. SWS was positively associated with anti-inflammatory serum complements (C1INH and CFI) and was negatively associated with pro-inflammatory serum C-4 (Table 3), which is in agreement with the theme that poor sleep is associated with increased pro-inflammatory markers and suppressed anti-inflammatory serum cytokine levels [34].

Awake time comprises SOL and WASO and is a negative indicator of sleep quality. In contrast to the report of Taheri et al., 2004, a negative curvilinear relationship between TST and BMI was found (Table 3), which might be due to differences between the samples of the two studies, such as age (almost 3 decades), BMI (approximately 6 kg/m²), gender composition, and the presence of chronic diseases, e.g., diabetes and hypertension [24].

The relationship between sleep and inflammatory cytokines is affected by gender [11,35]. Likewise, disease linked differences in sleep and inflammatory cytokines relationship have been previously documented [11]. The findings of this study provide primary evidence of polysomnographic correlates of inflammatory complement components in healthy young males. Future studies should explore the dynamics of this association between objective sleep measures and inflammatory complement components among females and across different age groups and comorbidities.

The limitations of our study include a relatively small sample size and that serum samplings for inflammatory complement components and other inflammatory mediators were performed at one time point (before subjects went to bed). The serum levels of inflammatory molecules, e.g., IL-6, C-3, and C-4, exhibit distinct circadian patterns in healthy individuals [14,36]. The blood sampling was done during a close range of time to avoid the effect of the circadian variability on the serum levels of the measured inflammatory molecules. Therefore, observation of the serum levels of inflammatory molecules at multiple time points may provide pertinent information about the circadian rhythm-dependent dynamics of their relation to sleep. However, the use of catheters for repeated blood sampling increases IL-6 locally [37]. Therefore, future studies should account for this fact to provide a comprehensive understanding of the association. Nevertheless, this is the first study to report that the inflammatory mediators across three classes of immune-regulatory molecules of cytokines, cell adhesion molecules, and complement components are similar in their associational dynamics with sleep. This study is a preliminary study to investigate the relationship between objective measures of sleep and inflammatory complement components. The normal level of oxygen desaturation index (4.33 ± 2.93) and mean SpO₂ (95.90 ± 0.83) indicated absence of apnea in the participants [38].

The findings of this study suggest a need for sleep evaluation in

health conditions where complement components are affected. Future studies should investigate sleep under conditions of complement component deficiencies and increased production (acute/chronic and local/systemic). More research is necessary to understand the obverse facet, i.e., the serum levels of complement component in sleep disorders. The application of anti-inflammatory medicines that affect the production and/or serum availability of inflammatory complement components (C-3, C-4, Factor-H, CFI, and C1INH) and/or inflammatory mediators (IL-6, sICAM-1) for the prospective management of sleep in patients suffering from chronic and/or systemic inflammation should be investigated.

5. Conclusion

Poor sleep measures in general were associated with heightened serum pro-inflammatory complement components and decreased anti-inflammatory complement components.

Conflicts of interest of each author/contributor

Md. Dilshad Manzar, received fellowship grant from Indian Council of Medical Research (3/1/JRF-2007/MPD), M. Ejaz Hussain received major research grant from the University Grants Commission (Grants no. -37-222/2009 (SR-)) and Ahmed S. Bahammam received grant from the Strategic Technologies Program of the National Plan for Sciences and Technology and Innovation in the Kingdom of Saudi Arabia. These funding agencies were not involved in the study design, data collection and/or result interpretation. S.R. Pandi-Perumal is a stockholder and the President and Chief Executive Officer of Somnogen Canada Inc., a Canadian corporation. He declares that he has no competing interests that might influence the content of this article. Other authors (Abu Hasnath M Golam Sarwar, Mohd. Shoeb Alam, Majumi M Noohu, Wassilatul Zannat) report no issues with conflict of interest. Finally, all authors declare that they have no proprietary, financial, professional, or personal interest of any nature or kind in any product or services and/or company that could be construed or considered to be a conflict of interest that might have influenced the views expressed in this manuscript.

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