**ORIGINAL ARTICLE**

# The Role of Cytokines in Depression and Sleep Regulation

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## ABSTRACT

**Background:** There is an interrelationship between the biological clock, the nervous system and the immune system in college students. Psychological and behavioral factors impact the immune system and this changes affect the depression and neural status of the individual.
**Methods:** All students who entered the Shiraz University of Medical Sciences from 2016-2017, were potentially eligible to participate in this randomized field trial. The sample size was 152 students divided into 2 groups: Sleep and wakefulness workshop and Control group. Finally, 119 of 156 participants entered in the study, Beck depression inventory and Pittsburgh sleep Quality Index.
**Result:** Based on the results, comparison of depression in the intervention group before and after workshop, had a significant decrease. For PSQ analysis, intra-group comparisons in those who participated in the sleep and awakening workshop showed that the change was significant. Our findings showed that in our participant, depression and sleep quality had a positive and significant relationship with Human IL-1β, Human IL-8 and Human IFN-γ.
**Conclusion:** Based on the findings of the Psycho-Neuro-Endocrine-Immunology, there is a reciprocal relationship between the biological system and sleep (based on the biological clock), the nervous system and the immune system and the changes in the immune system affect the mental and nervous conditions of the individual.
**Keywords:** Depression , Cytokines, Sleep.

## INTRODUCTION

Stressful experiences can bring on depression and stress-induced changes in physiology and trigger immune component[1,2]. According to the reports by National Mental Health Association, 46% of male students and 64% of female students suffer from mental disorders. The importance of depression among college students due to expansion of this disorder from initial stages of life to next stages, creates compliance problems, and crime in the future[3]. Based on the depression literature while "sickness behavior" signifies the transient and adaptive response to immune activation, "depression" refers to chronic and maladaptive response to immune activation, specifically chronic inflammation or stress. Ideally, both psychiatric rating scales and biological factors should support a diagnosis of depression, as it is the case with other medical conditions and disorders[4]. In addition, it has been determined that many factors may trigger inflammation and affect the development of depression embrace medical illness, psychosocial stress and sleep disorder[5].

It has been shown that immune system gives normal responses to interventions used to treat depression and related psychological disorders. Mounting evidence exists that inflammatory cytokines are involved in the development of neuropsychiatric symptoms and depression. Peripheral cytokines can access the brain and activate local CNS inflammatory networks to produce alterations in neurotransmitter function. numerous biomarkers and gene polymorphisms that confer vulnerability to cytokine-induced depression have been identified, and may be useful in identifying at-risk patients. There is a wide-range of immunologic and neurobiological mechanisms that may explain cytokine induced depression, many of the proposed therapeutic interventions are in conceptual or pre-clinical stages[6].

It has been shown that human biomarkers linked with cytokine-induced depression, include pro inflammatory cytokines and a dysregulation of immune mediators, nitric oxide and glucocorticoids and related symptoms of anorexia, sleep disorder, fatigue, and cognitive impairment. Moreover, exposure to a psychosocial stressor can augment the impacts of immune activation on sickness, plasma corticosterone and hippocampal norepinephrine, and on the levels of circulating cytokines. The active role of molecular events actuating synaptic transmission, neuronal plasticity, and depressive behaviors has been confirmed[7,8].

Since the studies done on the mental health of the students have been mainly about describing the mental health status of the students or identifying the factors that have to do with the students' mental health, it seems like that conducting researches on the effect of interventions on physical- mental health is needed.

## MATERIAL AND METHODS

In this randomized controlled field trial study, all of the students who entered to Shiraz University of Medical Sciences in the time of interval (2016-2017) were potentially qualified.

Participants in this study consisted of young college students and they were aware of the stages of the research. In this study, 156 participants scheduled in the first phase which were categorized randomly into two groups including sleep and wakefulness workshop (study) and control group. Participants were randomly assigned to equal groups. The study group participants underwent weekly intervention which was conducted by the intended professors and specialists of this research to reduce their stress. The participants were interviewed weekly and three months later in order to track the given training and the progress, the necessity, and the process of intervention were studied.

The control group continued their normal lives. However, they were interviewed every three months to record all the important lifestyle changes. Immunological status were checked as follow: Human IL-1β, Human IL-8 and Human IFN-γ.

In the experiment phase, 156 blood samples were taken and the results were reported to each person secretly according to the code of each person. However, due to the high cost of kits for immunological tests and the loss to follow up during this period, these tests were not carried out and the students' sera were frozen at a temperature of -20 ° C from February to November 2017 in the Immunology Department Laboratory. After nine months, the participants who had completed all of the questionnaires and actively participated in the workshop or related learning programs from the initial phase were invited. For the longitudinal analyses, of 156 participants, thirty-seven participants had missing data on all relevant variables and they were excluded from the study in November 2017 for many reasons including: disease and health problem (one participant), transferring to another university (one participant), and emotional problems (two participants), not willing to attend the final stage of blood sampling (27 participants), and unknown variables (six participants). Eventually, 119 out of 156 participants were remained. In the 9th month, we conducted the final immunological tests for two stages on blood samples of 119 participants who completed the follow-up until the last stage, completed all the questionnaires and participated in the final blood sampling. At the same time, the volunteers' mental health characteristics, stress level and sleep/wakefulness status were determined through interviews and using related questionnaires as follows: Beck depression inventory and Pittsburgh sleep Quality Index.

**Data Analysis:** Descriptive and inferential statistic was analyzed using SPSS 21; Pearson Correlation Coefficient was used to determine the relationship between variables and stepwise regression analysis was used to determine the variation level of variance of dependent variable. Data analysis was performed using Chi- square test for comparing the qualitative variables among the two groups and One-Way ANOVA was used for comparing the quantitative variables. In within-group analysis for comparing parameters before and after the intervention, the paired t-test was used. In all the tests, the significance level was considered as $p < 0.05$.

## RESULTS

Participants in this study consisted of young college students aged 18, 19, and 20. The age distribution of individuals is presented in Table 1 which is based on the comparison of the two studied groups. The results of Chi-square test showed that there existed no statistically significant difference in age of individuals among the two groups and the two groups were similar in terms of age. The mean age of the students including both male and female was 18.60 ± 0.793 years.

Both sexes were presented as the study samples, including 111 females (71.2%) and 45 males (28.8%), that were randomly selected in the 2 groups. 92 participants (59%) live in their own family and 64 participants (41%) live without family in the dormitory that were randomly selected in the 2 groups. The "sex and how to live with family" distribution of individuals is presented in Table 2.

Comparison of the two studied groups showed that there existed no statistically significant difference of individuals among the two groups and the two groups were similar in this terms. Based on the findings, there was no significant difference between the study groups in pretest descriptive indices hence, the study groups were homogenous in terms of the mentioned factors.

In other word, there was no significant difference between the study groups regarding the demographic variables and questionnaire scores, and the groups were matched by the mentioned variables. Regarding the demographic data, no significant difference was reported between the groups in pretest assessments. Overall, these findings suggest that no significant differences do exist based on demographic group membership.

To compare the scores of sleep (PSQ) and depression (DEP) statuses among the two groups, ANOVA test was used. Also, the changes between the pre and post intervention scores in two groups were determined using this test. In within-group analysis, paired t-test was used for comparing the scores before and after the intervention. The results of ANOVA showed that the difference in the observed changes among the two groups was not significant at PSQ but the differences in the DEP domain was significant. This difference is due to the differences between the groups of study and control ($P<0.001$ in both domains). In intragroup analysis, the changes seen in the two groups were very significant. However, in the DEP domain, although the changes in the control group were also significant, the rate of change is to increase the score of Dep. However, in the two groups, the amount of changes is significant and decreasing (Table 3).

To investigate the relationship between the interleukins and the scores of PSQ and Depression, the Pearson correlation coefficient test was used. The results are summarized in the form of correlation coefficient and probability number in Table 4. As observed, there is a significant and direct relation between PSQ and Depression with all of the interleukin parameters. For example, increases in the levels of Dep leads to an

increase in the levels of IL. Also decrease in sleep quality (increase PSQ score) leads to increase in the levels of interleukins.

Table 1: Participant Demographic Characteristic (Age) in 2 groups

| Age in years | Study group | Control group |
|---|---|---|
| 18 | 31 (59.6) | 30 (57.7) |
| 19 | 11 (21.2) | 13 (25) |
| 20 | 10 (19.2) | 9 (17.3) |

Statistic: 0.634, P value 0.959

Table 2: Demographic (Sex and Living Characteristics) in all Samples

| Variables | Frequency | %age |
|---|---|---|
| **Gender** | | |
| Male | 111 | 71.2 |
| Female | 45 | 28.8 |
| Total | 156 | 100 |
| **How to live with family** | | |
| With family | 92 | 59 |
| Without family | 64 | 41 |
| Total | 156 | 100 |

Table 3: Comparing Between 2 Groups in Pittsburgh Sleep Quality (PSQ), and Depression (DEP ) Parameters

| Variables | Study Group | Control Group | Statistic | P-Value |
|---|---|---|---|---|
| PSQ | | | | |
| Baseline | 7.15±4.46 | 7.26±4.53 | 0.010 | 0.990 |
| Final | 7.72±4.18 | 7.44±3.42 | 0.418 | 0.659 |
| Change | 0.15±2.13 | 0.44±2.12 | 2.20 | 0.115 |
| Statistic | 0.44 | 1.29 | | |
| P- value | 0.659 | 0.203 | | |
| DEP | | | | |
| Baseline | 12.48±9.50 | 11.85±9.86 | 0.078 | 0.925 |
| Final | 11.15±9.44 | 12.97±9.88 | 0.442 | 0.644 |
| Change | -1.77±1.16 | 1.13±2.20 | 44.5 | <0.001 |
| Statistic | 9.63 | 3.16 | | |
| P- value | 0.001 | 0.003 | | |

Table 4: Correlation Coefficient Between Interleukin and PSQ and Depression

| Variables | PSQ | | Depression | |
|---|---|---|---|---|
| | Coefficient | P-Value | Coefficient | P-Value |
| IL-1β (pg/mL) | 0.97 | <0.001 | 0.489 | <0.001 |
| IL-8 (pg/mL) | 0.501 | <0.001 | 0.412 | <0.001 |
| IFN-γ (pg/mL) | 0.598 | <0.001 | 0.494 | <0.001 |

## DISCUSSION

Understanding the relationship between sleep and wakefulness and its impact on mental health, the student's immune system and Psycho-Neuro-Endocrine-Immunology requires a lot of research. Studies have shown that sleep deprivation affects physiological and psychological function and enhances the energy of the organs of the body. Sleep is a recovery process for the immune system, in contrast, increased stress and depression disrupt the mechanisms of defense and immunity. In this study, workshop and education was considered as a good predictor of sleep quality in college students. Generally, adequate rest, sleep and comfort, are the main responsibilities of healthcare providers. Precise assessment of patients' previous sleep habits is very important for their mental and health[9,10]. Our results revealed presence of high prevalence of poor sleep quality among case study groups. This may be attributed to numerous activities and stresses facing college students, which may necessitate excessive study during night. This rate coincides with two recent studies from Pakistan[11] and Spain[12]. Such high rates of poor sleep among college students from different countries requires great concerns for dealing with stresses facing medical students. This can be improved through counseling and regular education to improve behavior and lifestyle. On the other hand, lower rates of poor sleep were reported from older studies from the USA[13] and Lithuania[14]. This discrepancy may be attributed to the differences in sample sizes, race, target population, or the time of conduction of the studies. In addition, nowadays there is a marked increase in the night use of social media, which may increase the percentages of poor sleepers. However, this may be attributed to higher prevalence of psychologic problems as depression among college students, and the association between these problems and sleep disturbance[15].

Younger students and those enrolled in the basic educational years in the current study had higher rates of poor sleep quality compared to comparative partners and enrollment in the basic year was the one predictor of poor sleeping. Anxiety and the pressure of passing the first years of medical school might also play role[16]. This finding coincides with results of a study of Brick et al. from California University, USA[13]. It agrees also with results of a recent study, 2017, from Egypt[17]; as students enrolled in the preclinical years obtained higher score of PSQI compared to others. On contrary, the Indian study revealed a significant positive association between age and poor sleep quality. This inconsistency of results may be because the Indian study was conducted among undergraduates, interns and post-graduate physicians. The latter two groups may have more load, stresses, and nigh on-calls. This may precipitate poor sleeping; as doctors may suffered from acute sleep deprivation during their on-calls[18,19].

In the current study, depression was the predictor of poor sleep quality, which is in line with the results of studies from India[19] and Ethiopia[20]. Furthermore, students who were categorized as having morbid depression were more poor sleepers than others. This finding coincides with results from Virginia[20], Ethiopia[19] and Egypt[16]. There is an evidence of association between depression and insomnia and hence with poor sleeping.

Coping and the ability to overcome negative emotions, is a reflection of a procedure including active involvement in a specific time period and has different strategies. Whatever the capacity to cope with stress and life challenges increases, a person can better maintain his mental and social health, and try to solve his problem in a positive, adaptive, and efficient manner[21].

In our study, the level of inflammatory cytokines was measured. We find that depressive symptoms predicted their elevations, potentially making individuals with depressive symptoms more prone to inflammatory diseases. Our findings suggest that, in our sample, depressive symptoms have a positive association with cytokines. Our longitudinal findings are consistent with

earlier cross-sectional findings demonstrating a relationship between depression and inflammation[22]. Our findings suggest that, in our sample, depression and sleep quality have a positive association with cytokines. Our longitudinal findings are consistent with earlier cross-sectional findings demonstrating a relationship between these factors, depression and inflammation[23,24].

It was deduced that cytokines both signal the brain and act as mediators between the immune and central nervous systems. Neuropsychiatric functions like mood and cognition in part are under the influence of cytokines and other immune molecules through their modulation of neuronal anatomy and function. Neuronal plasticity is responsible for normal regulation of mood, cognition, and behavior across the lifespan. Neuronal plasticity impairment which has a hand in cognitive and mood disorders can be caused by cytokines and other immune factors[25].

Based on numerous studies performed on patients with idiopathic major depression, cytokine concentrations have been investigated and measured in many studies and some have observed the correlation of increased cytokines with depression severity. A decreased potentiality for glucocorticoids to inhibit inflammatory cytokine production has been observed in depressed patients. Furthermore, in adolescents with histories of childhood adversity, increased cytokines production has been observed to precede the development of depression 6 months later, suggesting a relationship between chronic stress, inflammation, and depression[26,27].

More recently, there has been increasing recognition of the role of the innate immune system in depression. The involvement of the innate immune system in the response to stress is consistent with the finding that the innate immune system is abnormal in the context of chronic stress and demonstrates over-activity in depressed individuals, confirmed in meta-analyses looking at peripheral levels of immune proteins[28,29].

Generally, our sample included college students, educated, healthy adults with less depressive symptoms so it may underestimate the effects of depression on inflammation and vice versa. Our study provides novel evidence the relationship between depression and later inflammation via complex ways. More specifically, who had low levels of depression had lower levels of inflammatory cytokines. Conversely, those who utilized higher immunological levels, had higher levels of depression. This finding suggests that higher use of intention to when depressed exacerbates interleukin production possibly due to chronic emotional dysregulation or the use of problem solving.

## CONCLUSION

Cognition within determination is associated with some changes in the immune system. It has been shown that immune system gives normal responses to interventions used to treat depression and related psychological disorders. Factors that may trigger inflammation and affect the development of depression embrace medical illness, psychosocial stress, sleep changes and sleep disorder. Leading to a series of positive emotions and feelings and they are factors that can change the viewpoints and perceptions of people from the problems of life and make it easier to pass them in many cases. Combination of these components are helpful for our health through the immune system.

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