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Title: Sleep architecture and hypothalamic–pituitary–adrenal activity in paradoxical and psychophysiological insomnia

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Abstract

There are controversial reports about association between sleep and hypothalamic–pituitary–adrenal (HPA) activity. Studies have reported the influence of insomnia on HPA hormones. However, they usually ignored the heterogeneity of insomnia symptoms, so subtypes of the disorder have not been considered in the reports. The aim of the present study was to investigate the final and intermediate products of HPA system among a group of psychophysiological and paradoxical insomniac patients in comparison to a group of normal sleepers. We investigated the awakening serum level of adrenocorticotropic hormone (ACTH) and cortisol after one night polysomnography (PSG) in 17 subjects with psychophysiological insomnia, 19 subjects with paradoxical insomnia and 17 subjects with normal sleep profile. Groups were matched for age and body mass index (BMI). Serum levels of ACTH and cortisol were measured by enzyme-linked immunosorbent assay (ELISA) method. Although, a tendency toward elevation of both ACTH and cortisol was observed among patients with paradoxical insomnia compared to both control and psychophysiological insomnia, but the results were not significant comparing three groups. According to regression analysis, higher level of ACTH was significantly predicted by higher NREM arousality and pulse transit time (PTT). These findings could suggest the personality traits hypothesis for paradoxical insomnia. Both cortical and subcortical arousality could lead to more HPA activity and higher ACTH level. Further studies are recommended to confirm the hypothesis.

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Keywords: cortisol, adrenocorticotropic hormone (ACTH), sleep disorder, insomnia

1. Introduction

Sleep is an important body homeostatic behavior that plays a critical role in emotional and cognitive functions (Koren et al., 2011). Poor sleep quality could disrupt the endocrine system (Ioja, Weir, & Rennert, 2012; Mullington, Simpson, Meier-Ewert, & Haack; Potter et al., 2016). More specifically, sleep duration and sleep quality could alter biological systems responsive to stress (P. Meerlo, Sgoifo, & Suchecki, 2008). Biological rhythms that regulate two main stress systems; including hypothalamus-pituitary-adrenal (HPA) and autonomic nervous system (ANS), are controlled by suprachiasmatic nuclei (SCN) of hypothalamus. The SCN as a circadian pacemaker, also responsible for modulation of sleep-wake cycle (Hastings, Reddy, & Maywood, 2003). Previous studies have emphasized that the circadian mechanisms which control the sleep, also directly modulate the ANS (Leproult & Van Cauter, 2010). Sleep disturbances change the autonomic activity and lead to increased heart rate and blood pressure (Zhong et al., 2005). In addition, brain centers control sleep-wake cycle and stress-responsive biological systems are closely interacted. Therefore, sleep duration may change the patterns of stress systems (Edwards, Evans, Hucklebridge, & Clow, 2001).

Insomnia is the most prevalent sleep disorder that affects 10% of adult population (Morin & Benca, 2012; Ohayon & Reynolds, 2009). It is identified as difficulties in initiating and maintaining sleep, or early-morning awakening with inability to return to sleep. It should occur at least three times per week, and produce clinically significant impairment in social, occupational or other aspects of functioning (APA, 2013). Despite the high prevalence of insomnia and its wide

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health and economic outcomes, the biological mechanisms of bidirectional interaction between stress systems and sleep have not been fully understood.

Although there is a physiological link between neural representation of sleep-wake cycle and stress-responsive systems, inconsistent results have been reported about association between sleep architectures and cortisol level as a final product of HPA (Elder, Wetherell, Barclay, & Ellis, 2014). Association between sleep duration and awakening cortisol level has been reported. In a study about association between sleep, stress and cortisol awakening response that conducted on 58 normal adults, lower subjective total sleep time was significantly linked to lower cortisol levels at awakening (Vargas & Lopez-Duran, 2014). Similarly, reduced cortisol level after 24 h sleep deprivation was reported (Arnal et al., 2016). In contrast, increased serum cortisol level has been reported among people with insomnia in many previous studies (Floam et al., 2015; Peter Meerlo, Sgoifo, & Suchecki, 2007; A. Rodenbeck & Hajak, 2001; Andrea Rodenbeck, Huether, Rüther, & Hajak, 2002). Some studies failed to indicate an association between sleep duration and cortisol level. Federenko et al reported a relation between awakening time and cortisol level but no association between sleep duration and this adrenocortical hormone (Federenko et al., 2004). However, some studies didn't indicate an association between sleep and HPA axis activity at all (Lattova et al., 2011; Varkevisser, Van Dongen, & Kerkhof, 2005). These different results that ranged from positive or negative association to no relation between sleep and HPA activity emphasize the need for more investigation in this area. More investigations by well-control equipment for objective analysis of sleep architectures would helpful for understanding these discrepancies.

Broad controversies about the association of sleep and HPA activity indicate that this association is not a static unidirectional relation but rather it is a dynamic

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interaction that is modulated by different aspects of sleep characteristics. A recent study indicated that sleep deprivation caused higher morning cortisol levels, whereas sleep misalignment lead to lower morning cortisol levels (Wright et al., 2015). In addition, research for association between sleep and HPA activity among patients with insomnia revealed the inconsistent results. Although some studies reported an increased serum cortisol level in people with insomnia (Floam et al., 2015; Peter Meerlo et al., 2007; A. Rodenbeck & Hajak, 2001; Andrea Rodenbeck et al., 2002) , but some other studies didn't find a significant association (Dahlgren, Kecklund, Theorell, & Akerstedt, 2009; Eek, Karlson, Garde, Hansen, & Orbaek, 2012; Hsiao et al., 2013; Riemann et al., 2002). Using subjective self-reported questionnaires or objective laboratory-based equipment may lead to different findings. In some studies self-reported insomnia symptoms have been associated with lower waking cortisol. For example, Backhaus et al. (2004) reported a significantly lower cortisol after awakening in patients with primary insomnia compared to control. They reported negative correlation between subjective sleep quality measured by Pittsburgh Sleep Quality Index (PSQI) and salivary awakening cortisol (Backhaus, Junghanns, & Hohagen, 2004). Also it has been reported that sleep problems during the past month were associated with low morning and evening salivary cortisol level (Hansen et al., 2012). In other hand, significant association between objective sleep characteristics measured by actigraphy and diurnal cortisol patterns has been reported (Castro-Diehl et al., 2015). Different characteristics of sleep and different methods for investigating sleep architectures may explain controversial reports. Previous studies have usually considered the sleep duration and quality for investigation the association between sleep and HPA. Nevertheless, there are important sleep EEG characteristics and polysomnography (PSG) data on related physiological system that may generate more finding on the relationship between HPA and sleep homeostasis. Our

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knowledge about association of sleep architecture and sleep EEG structures with HPA activity is limited. In another recent study, up-regulation of cortisol was associated with wake after sleep onset using actighraphy and diary based sleep monitoring (Floam et al., 2015). According to this finding, sleep fragmentation may be a more potent factor to influence HPA activity compared to total sleep time and sleep efficiency. Therefore the PSG as a more suitable method for detection of the breakup events during sleep may appropriately reveal interactions between sleep and HPA activity.

Another important problem in studies of insomnia is ignoring the subtype of the disorder. Inconsistent findings about association between sleep and HPA activity may arise from the fact that insomnia is a symptomatically heterogeneous disease (Floam et al., 2015). Previous studies reported the HPA data on insomnia but data about subtypes of the disorder such as paradoxical insomnia is rare. Paradoxical insomnia as a sleep state misperception condition is defined as an insomnia without objective findings. This subjective but not objective insomnia, is a subtype of primary insomnia, with prevalence ranging between 9.2% and 50% of patients with insomnia (American Academy of Sleep Medicine AASM, 2005). Patients with paradoxical insomnia report little to no sleep over long periods of time, but objective sleep findings such as PSG near-normal sleep patterns in these people (~~J. D.~~ Edinger & Krystal, 2003). Additionally, they do not display the level of daytime sleepiness that experienced after sleep deprivation (American Academy of Sleep Medicine AASM, 2005). Considering the PSG and actigraphy results that paradoxically are similar to normal sleeper in spite of insomnia complaint, the HPA activity would be a discriminative index for distinguishing paradoxical insomnia from normal sleeper and psychophysiological insomnia. An important etiological hypothesis suggests the role of personality traits in paradoxical

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insomnia (Dorsey & Bootzin, 1997; Vanable, Aikens, Tadimeti, Caruana-Montaldo, & Mendelson, 2000). However, there is no consensus about why these personality traits may play causal role in paradoxical insomnia (Dorsey & Bootzin, 1997). It has been suggested that these traits may lead to higher anxiety level and subsequent misperception of sleep (Harvey & Tang, 2012). Therefore, the study of biological systems responsible for stress could test this hypothesis and reveal associations between sleep misperception and stress system.

In the present study, we aimed to investigate the serum levels of final and intermediate products of HPA system among a group of psychophysiological and paradoxical insomnia patients in comparison to a group of normal sleeper. In addition, associations between HPA activity and objective sleep architectures as well as autonomic activity were investigated.

2. Materials and methods

2.1. Participant

Thirty-six consecutive patients with insomnia complaint who were referred to Sleep Disorders Research Center (SDRC) of Kermanshah University of Medical Sciences (KUMS) from April 2014 to October 2016 participated in the study. They included 21 females (58.3%) and 15 males (41.7%) aged 14 to 62 years (41± 12.2). Twenty one subjects with normal sleep consisted of 4 females (19%) and 17 males (81%) with the age range of 26 to 59 years (40.7±10) were invited from Kermanshah province as control group. They were matched to insomniac groups for age and BMI. The study was approved by the Ethics Committee of KUMS and all participants completed detailed written informed consent. Any of the following condition led to exclusion of participants from the study; chronic medical disorders, other sleep problems, any substances use, neurological disorders, psychiatric conditions, respiratory problems and cardiovascular disorders.

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Medications such as benzodiazepines that may affect sleep characteristics were stopped for 2 weeks before data collection. Other sleep conditions such as hypersomnias, parasomnias, circadian sleep-wake disorders, and restless legs syndrome identified by physician's examinations, and sleep breathing and periodic limb movement disorders identified by PSG were also excluded from the study.

2.2. Insomnia Diagnosis and subjective sleep investigation

The insomnia was diagnosed by sleep clinician according to ICSD2 based clinical interview (American Academy of Sleep Medicine AASM, 2005). At the first step, all insomniac participants and normal participants were clinically interviewed by an experienced psychiatrist who had fellowship in sleep medicine according to international classification of sleep disorders-2nd edition (ICSD2) (American Academy of Sleep Medicine AASM, 2005) criteria. Participants in insomnia group were referred to the second step for PSG investigation if they diagnosed as insomnia according to ICSD2. In addition volunteers in normal control group were also interviewed and those who had not any sleep disorder were selected for the second step of the study. Subjective diagnostic criteria for insomnia included; 1) A subjective complaint of insomnia characterized by difficulties initiating and/or maintaining sleep; 2) Insomnia must have been present at least three nights a week for more than six months; 3) A complaint of at least one daytime consequence attributed to insomnia; 4) Distress or significant difficulties in social and/or occupational functioning; 5) A subjective sleep efficiency (SE) below 85% in their two week sleep diary prior to PSG recordings. Four participants in the control group were excluded from the study due to symptoms of obstructive sleep apnea (OSA) and did not go to the next step according to the first interview. In the next step, participants were invited to performing a whole night PSG procedure. The

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inclusion and exclusion criteria for both patients and control groups were confirmed by PSG results.

PSQI was used for subjective sleep investigation (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). Sleep quality over the past month was measured by PSQI in a self-reported manner. The questionnaire consists of 19 individual items that creating 7 components including sleep quality, sleep latency, total sleep time (TST), sleep efficiency (SE), sleep disturbances, the use of sleeping medication, and the daytime dysfunction. We considered subjective TST, SE and sleep latency for analysis in the present study.

2.3.PSG procedure

To recording the sleep physiological parameters, participants performed an overnight polysomnography (SOMNOscreen plus®, Somnomedics, Germany). Participants were invited to sleep laboratory of SDRC and an appointment was scheduled according to the participant's desire. They advised to don't have any snooze and sleep during the day before the appointment. In addition they should avoid coffee, tea, heavy diet and cigarette as well. They should arrived the laboratory at 21 pm. Then, participants completed the demographic and PSQI questioners. PSG procedure was explained for participants and any question was answered. PSG room was standardized for any noise and visual stimulus based on international standard (American Academy of Sleep Medicine AASM, 2002). Recording of PSG was started based on the subject's usual sleeping habits, and each patient was recorded for a minimum of 7 hours.

American Academy of Sleep Medicine guideline was considered for measurement of PSG (American Academy of Sleep Medicine AASM, 2002). Electroencephalogram was recorded using frontal, central and occipital leads

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according to 10-20 system. Also, electro-oculogram (EOG), electromyogram (EMG), oximetry, abdominal and thoracic respiratory effort (induction plethysmography), and body position were recorded. Oronasal thermocouples and nasal pressure transducers were used for monitoring the respiration. Continuous pulse oximetry was also monitored and thoracoabdominal movements were monitored by piezoelectric strain gauges.

Sleep latency of less than 15 minutes, SE of more than 85% and TST of greater than 7 hours were considered as objective normal sleep pattern for normal control group. The presence of marked discrepancies between subjective and objective sleep measures (i.e. a difference of one hour or more for TST, or a difference of at least 15% between subjective and objective measures of sleep efficiency), an objective TST of more than 6 hour and 30 min, and a SE greater than 85% on nocturnal PSG were considered as criteria for diagnosing of paradoxical insomnia (~~Jack D~~ Edinger et al., 2004). Finally, patients with subjective insomnia diagnosis that failed to reach the above mentioned criteria and didn't have other sleep conditions considered as psychophysiological insomnia. TST, SE, sleep latency, non-rapid eye movement (NREM) sleep stages, total REM percent, total REM arousality, total NREM arousality, wake index, wake after sleep onset (WASO) and pulse transit time (PTT) were extracted and considered for further analysis. PTT is a time needed for receiving a pulse wave from heart to the finger. It has been considered as a blood pressure marker and related to ANS activity (Hey & Sghir, 2011).

2.4. Biochemical analysis

Five milliliters of venous blood samples were collected at 8 am after PSG recording. Serum was separated by centrifuge and stored at -20ºC for the biochemical analyses according to standard protocols.

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Serum levels of cortisol (Code 3625-300; Monobind Inc.) and ACTH (REF 7023, BIOMERICA) were measured by using enzyme-linked immunosorbent assay kits. Awareness Technology STAT FAX 2100 Microplate Reader (Awareness Technology, USA) was used for reading the level of absorbance. Hormone levels were calibrated to the standard calibration curve of each hormone according to the kits. Data were presented as pg/mL for ACTH and μg/dl for cortisol.

2.5. Statistical analysis

Data were analyzed between psychophysiological insomniac, paradoxical insomniac and control groups by Chi-square and ANCOVA. Significant differences between groups was detected by post hoc Tukey multiple comparisons. The levels of hormones and sleep characteristics were compared between three groups and the age, gender and BMI were used as covariates. Association between PSG sleep characteristics and biochemical parameters was evaluated by multiple linear regression analysis according to covariates of age, gender, BMI and group. All model assumptions were evaluated by residual analysis. The statistical package for social sciences (SPSS) (SPSS, Inc., Chicago, IL) version 16.0 was used for the statistical analysis.

3. Results

3.1. Demographic findings

We recruited 57 subjects with the mean age of 41.52±11.3 years. Four participants in the control group were excluded from the study due to OSA diagnosis in the first psychiatric interview. Finally, 53 subjects with the mean age of 40.92±11.5 were included in the study. In the normal control group, 17 participants completed the study. According to PSG criteria patients were divided

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to 19 subjects including 13 females (68.4%) with paradoxical insomnia (32-53 years; 43.2±6.4) and 17 subjects including 8 females (47.1%) with psychophysiological insomnia (14 to 62 years old; 38.4±16.3 years) (table 1).

Demographic characteristics of participants are presented in Table 1. Three groups were age and BMI-matched. However, there are significantly higher numbers of females in paradoxical and psychophysiological insomnia groups. We considered the sex as a covariant in all analysis.

Table 1. Demographic characteristics of studied groups

	Normal (n=17)	Paradoxical insomnia (n=19)	Psychophysiological insomnia (n=17)	*p*-value
Age†	40.76(10.1)[a]	43.26 (6.45)[a]	38.47 (16.36)[a]	0.466[c]
Sex				
Female	4(23%)	13(68.4%)	8(25.0%)	**0.027**[b]
Male	13(76.5%)	6(31.6%)	9(52.90%)	
BMI	26.57 (3.82)[a]	26.55 (3.8)[a]	26.97 (6.54)[a]	0.961[c]

† Mean (standard deviation). Data compared by [c]ANOVA and [b] Chi-Square test. Means with the same superscript letters within a row are not significantly different (p > 0.05).

3.2. Objective and subjective sleep characteristics

We considered subjective TST, SE, and sleep latency obtained from PSQI for analysis. In addition, PSG results including; TST, SE, sleep latency, sleep efficiency, NREM sleep stages, total REM percent, total REM arousality, total NREM arousality, wake index, WASO, and PTT were considered for analysis. We adjust the sex, age and BMI in all analysis. According to PSQI results, TST, SE, and sleep latency were significantly different comparing three groups (p<.01). Post hoc analysis by Tukey test, indicated that subjective TST was significantly low in paradoxical insomniac group compared to normal sleepers (p<.001) and psychophysiological insomnia (p=.001). Also, in paradoxical insomniac group the

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subjective sleep latency was significantly higher than normal sleepers (p<.001) and psychophysiological insomnia (p<.001). In addition, subjective sleep efficiency in this group was lower than both normal sleepers (p<.001) and psychophysiological insomniac group (p=.002) (table 2).

Different results were obtained for objective sleep characteristics investigated by PSG. According to Tukey post hoc analysis test, psychophysiological insomnia indicated significantly lower objective TST compared to normal sleepers (p=.017) and a lower non-significant objective TST compared to paradoxical insomniac group (p=.073). Similarly, psychophysiological insomniac group had significantly lower sleep efficiency compared to normal sleepers (p=0.004) and paradoxical insomniac group (p=0.042) (table 2). Although, objective sleep latency was not significantly different comparing three groups, but it was considerably high among patients with psychophysiological insomnia compared to paradoxical insomniac and control groups (p=.071). The PSG wake index was significantly higher among psychophysiological insomniac group compared to normal sleepers (p=.014) and paradoxical insomniac groups (p=.04). Also, WASO was significantly higher among psychophysiological insomniac group compared to normal control (p=.001) and paradoxical insomniac group (p=.02) (table 2). Maximum, minimum and average PTT was lower in paradoxical insomniac group but the difference between groups was only significant in minimum PTT (p=.047). According to Post hoc analysis, the difference of minimum PTT between paradoxical insomnia and normal sleepers was not significant (p=.17). Similarly, there was no significant difference in minimum PTT between paradoxical and psychophysiological insomniac groups (p=.05) (table 2). A schematic representation of PSG sleep architecture in three studied group is presented in figure 1. As represented in the figure, N3 stage was decreased and REM stage was increased in paradoxical and

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psychophysiological insomniac groups compared to normal control group (figure 1).

Table 2. Sleep structures among normal sleepers and insomnia patients

	Normal (n=17)	Paradoxical insomnia (n=19)	Psychophysiological insomnia (n=17)	*p*-value
PSQI total sleep time† (h)	5.57(1.93) [a]	2.25(2.46) [b]	4.60(2.73) [a]	<.001
PSQI sleep latency (h)	.79(.57)[a]	3.01(1.24)[b]	1.39(1.32)[a]	<.001
PSQI sleep efficiency	74.78(25.96) [a]	28.88(20.49) [b]	58.28(32.66) [a]	.001
PSG total sleep time	7.11(0.44)[a]	6.82(0.69)[ab]	6.02(1.58)[b]	.015
PSG sleep latency	7.84 (6.60) [a]	9.13 (6.58) [a]	15.30(26.40) [a]	.071
PSG sleep efficiency	92.79(4.32)[a]	88.04(7.28)[a]	77.25(19.94)[b]	<.001
N1 stage (%)	32.44(16.39)[a]	32.56(16.54)[a]	32.60(13.51)[a]	.410
N2 stage (%)	21.67(9.19)[a]	20.21(10.27)[a]	16.28(9.33)[a]	.321
N3 stage (%)	30.65(18.39)[a]	23.94(15.12)[a]	19.17(16.82)[a]	.154
REM (%)	8.01(8.30)[a]	11.32(8.86)[a]	9.13(9.79)[a]	.816
Total REM arousality	20.50(11.05)[a]	23.86(12.62)[a]	24.31(18.28)[a]	.930
Total NREM arousality	24.76(9.02)[a]	24.74(6.26)[a]	22.99(5.43)[a]	.696
PSG wake index	2.02(0.68)[a]	3.54(1.92)[a]	8.92(10.81)[b]	.011
WASO	14.53(4.68) [a]	23.36(11.94) [a]	40.58(28.32) [b]	.001
Maximum PTT	360.93(22.20)	356.16(24.97)	361.71(19.94)	.73
Minimum PTT	262.97(10.02)	249.89(15.63)	266.18(28.92)	.047
Average PTT	305.93(10.78)	299.79(17.91)	308.24(14.72)	.23

†Mean (standard deviation). Statistical analysis for the equality of the mean values among the three groups was evaluated using ANCOVA adjusted by sex, age, and BMI ($P < 0.05$). Means with the same superscript letters within a row were not significantly different ($p > 0.05$). Pittsburgh sleep quality index(PSQI); polysomnography (PSG), rapid eye movements (REM), non-rapid eye movement (NREM) wake after sleep onset (WASO), pulse transit time (PTT)

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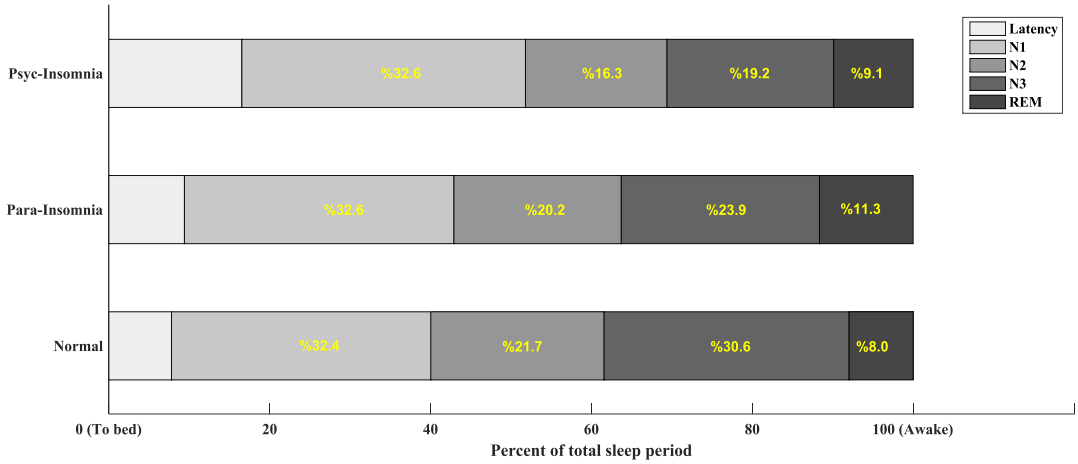

Figure 1. PSG sleep architecture among two subtypes of insomnia (psychophysiological and paradoxical).

3.3. Biochemical findings

All biochemical analysis was compared between groups by ANCOVA after adjustment of sex, BMI and age. According to the results, there was no significant difference between normal sleepers and two subtypes of insomnia regarding the biochemical parameters (Table 3).

Table 3. Biochemical parameters among studied groups

	Normal (n=17)	Paradoxical insomnia (n=19)	Psychophysiological insomnia (n=17)	*p*-value
ACTH	25.08(17.94)[a]	28.28(13.82)[a]	21.94(12.39)[a]	0.40
Cortisol	134.09(51.11)[a]	153.86(62.87)[a]	133.40(39.32)[a]	0.45

Statistical analysis for the equality of the mean values among the three groups was evaluated using ANCOVA adjusted by sex, age, and BMI ($P < 0.05$). Means with the same superscript letters within a row were not significantly different ($p > 0.05$).

Association between PSG sleep structures and biochemical parameters was analyzed by Linear Regression Model adjusted by sex, age, and BMI. According to results, total NREM arousality and maximum PTT had a significant effect on ACTH level (Coefficient=0.741; p=0.031). None of the PSG sleep characteristics influenced the cortisol level.

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Table 4. Multiple Analysis of Variables Associated with ACTH using the Linear Regression Model adjusted by sex, age, and BMI

		Coefficient	95% CI Lower	Upper	*p*-value
ACTH	Total Non-REM Arousality	.673	.029	1.266	.031
	Maximum Pulse Transit Time	.214	.006	.404	.043

CI, Confidence interval.

4. Discussion:

Insomnia is the most prevalent sleep condition that could disrupt body homeostasis and affect many physiological systems. Control of circadian rhythm and stress system in the brain overlap anatomically and physiologically. Both sleep circadian rhythm and stress-responsive biological systems are affected and regulated by SCN of hypothalamus (Hastings et al., 2003). According to previous studies, sleep-wake cycle and stress-responsive biological systems are closely interacted, so sleep duration may change the patterns of stress systems (Edwards et al., 2001). Therefore, the effect of insomnia on HPA has been suggested and investigated in methodologically different studies with inconsistent results. Ignoring the subtypes of insomnia and various sleep characteristics in previous studies motivated us to investigate the ACTH and cortisol, as intermediate and final products of HPA respectively, in two main subtypes of primary insomnia, including psychophysiological and paradoxical insomnia. To our best knowledge, the present study is the first investigation about HPA activity among people with paradoxical insomnia.

Results of present study didn't indicate any significant differences in ACTH and cortisol levels between normal sleepers and both subtypes of insomnia. However, both cortisol and ACTH levels among psychophysiological insomnia were non-significantly lower than paradoxical insomniac and normal sleeper groups. Previous studies indicated that shorter sleep durations lead to lower levels of

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waking cortisol in the next day (Van Lenten & Doane, 2016; Vargas & Lopez-Duran, 2014). In addition, subjectively-defined insomnia has been associated with normal (A. Rodenbeck et al., 2003; Andrea Rodenbeck et al., 2002; A. N. Vgontzas et al., 2001; Alexandros N. Vgontzas et al., 1998) or decreased morning awakening cortisol levels (Backhaus et al., 2004). In contrast, some studies reported up-regulation of cortisol among insomnia patients compared to controls (Floam et al., 2015).

According to PSG data, people with psychophysiological insomnia had significantly lower objective sleep duration and sleep efficiency compared to paradoxical insomnia and normal sleepers. So, lower awakening ACTH and cortisol levels were obtained, although the difference was not significant that may be due to low sample size.

In other hand, both ACTH and cortisol levels were non-significantly higher among people with paradoxical insomnia compared to normal group and patients with psychophysiological insomnia. According to present results, the psychophysiological subtype of insomnia doesn't have any significant effect on cortisol level as a final product of HPA. These findings suggest that psychophysiological insomnia may have not significant consequences on stress system. However, the condition for paradoxical insomnia might be completely different. paradoxical insomnia is a sleep state misperception condition (American Academy of Sleep Medicine AASM, 2005). Considerable mismatch between objective and subjective sleep architecture has been introduced as core diagnostic criteria for paradoxical insomnia (~~J. D.~~ Edinger & Krystal, 2003). PSQI subjective total sleep time and sleep efficiency was significantly lower among paradoxical insomnia compared to normal and even psychophysiological insomnia. Nevertheless, the PSG total sleep time and sleep efficiency were equal to normal group. Therefore, PSG results in group with paradoxical insomnia were similar to

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normal sleeper in spite of insomnia complaint. Future study with larger sample size about the effect of paradoxical insomnia on HPA activity may significantly reveal the role of stress system in this sleep misconception condition. The role of personality traits in the etiology of paradoxical insomnia has been suggested (Dorsey & Bootzin, 1997; Vanable et al., 2000). These traits may lead to higher anxiety level and subsequent misperception of sleep (Harvey & Tang, 2012). The higher anxiety level may reveal as a higher ACTH and cortisol levels, although the results of present study did not indicate a significant effect, which may be due to low sample size.

After control of the effects of age, gender, BMI and studied groups, one of PSG variables that could significantly affect the serum ACTH level is total NREM arousality. This index could be considered as an electrocortical arousal and sleep fragmentation sign. According to a previous study that used the subjective measure, higher frequency of awakening during night was directly correlated with awakening salivary cortisol level (Backhaus et al., 2004). Total NREM arousality could be considered as a good index of sleep fragmentation during slow wave sleep, the sleep stages that have a more recovery outcomes on brain physiology. We suggest that higher number of arousality during slow wave sleep may disrupt the refreshing effect of sleep on brain mechanisms related to HPA modulation.

Another variable that predict the ACTH level is maximum PTT. PTT is considered as a marker for blood pressure and could be related to ANS activity. It has been introduced as an appropriate measurement for stress (Hey & Sghir, 2011; Stefan et al., 2009). Therefore, the association between maximum PTT and serum ACTH level may be resulted from same neurophysiological mechanisms responsible for both PTT and ACTH and also the top-down higher control mechanisms modulating both HPA and PTT simultaneously. In addition the minimum PTT was significantly differ between groups and low among patients

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with paradoxical insomnia. It has been suggested that EEG arousal index couldn't indicate subcortical arousal events that modulate respiratory and blood pressure conditions. Therefore, PTT is a more suitable index for evaluating subcortical arousal that exerts vegetative and physiological consequences on stress system (Katz, Lutz, Black, & Marcus, 2003). Considering the PTT as a subcortical arousal may open a new window for investigation insomnia. Previous studies considered insomnia as a subjective judgment of short duration and less efficient sleep. By advanced technologies such as actigraphy and PSG, objective measurement of sleep duration and efficiency has been used in sleep studies. The EEG monitoring of sleep that records surface electrophysiological activity of cortex, but neurophysiological events such as reticular activating system (RAS) in the brain stem that contain important arousal modulator is ignored. So considering PTT as an index for subcortical arousality may provide important information for understanding the biological bases of insomnia.

5. Conclusion

People suffer from insomnia didn't indicate significant difference in awakening serum ACTH and cortisol levels compared to controls. Although, a tendency toward the elevation of both ACTH and cortisol levels was observed among patients with paradoxical insomnia compared to both control and psychophysiological insomniac groups. Further studies with large sample size are recommended.

Higher level of ACTH was significantly predicted by higher NREM arousality and PTT. Therefore, both cortical and subcortical arousality may lead to more HPA activity and higher ACTH level.

6. Limitation

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Some limitations should be taken into account when interpreting our results. The objective sleep duration in this study was based on one night PSG and may not be representative of habitual sleep duration and may be affected by first-night effects. In addition, we only measured awakening serum cortisol levels and we couldn't measure cortisol awakening response.

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