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Research Article

Melatonin Levels in Shift Nurses with ELISA and LCMS Methods, Jakarta, Indonesia

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ABSTRACT

A nurse has a high enough stress level because it is directly related to coping with various kinds of patients on shift work schedules. So far, the detection of stress cases is based on a questionnaire in which validity and relativity are quite good, although subjective, such as the Perceived Stress Score (PSS) questionnaire. This study aimed to examine objective markers of stress in shift working nurses by measuring melatonin levels.

Methods: The study was conducted from December 2019 to June 2020. The respondents were the shift and non-shift nurses from one of the public hospitals in Jakarta, Indonesia, by obtained through by consecutive sampling. Each group consisted of 40 people each. Melatonin was measured twice, at midnight on the last night shift (second night) in the shift group (pre-work), then at 08.00 am on the following day (post-work). The non-shift group was carried out on weekdays at 08.00 am pre-work and at 04.00 pm post-work. Measurements used Enzyme-Linked Immunosorbent Assay (ELISA) and Liquid Chromatography-Mass Spectrometry (LCMS).

Results: The percentage of nurses who showed moderate stress level in the shift group (30%) is higher than the non-shift (25%). There was a significant difference in the mean pre-work melatonin levels, measured with ELISA, between the shift and the non-shift group (51.53 pg/ml vs 17.07 pg/ml, P = ≤ 0,001), as well as the post-work examination (24.30 pg/ml vs 10.84 pg/ml, P = ≤ 0,001). We acquired a new reference for mean melatonin levels at 04.00 pm of 10.48 pg/mL (2.15-38.30 pg/mL) using ELISA and 38.13 pg/mL (5.00-230.00 pg/mL) using LCMS in non-shift nurses. In addition, a new reference for melatonin levels at 12.00 pm was 51.53 pg/mL (0.80-135.00 pg/mL) using ELISA and 52.35 pg/mL (10.00-170.00 pg/mL) using LCMS in shift nurses.

Discussion: This study showed differences in the results of measuring melatonin levels using ELISA and LCMS, which will be a good starting point for further research with a larger number of respondents. In addition, it is interesting to observe that a decrease in the mean level of melatonin from pre-work to post-work was found in both the nightshift and dayshift groups, using ELISA and LCMS.

Key words: Stress, ELISA, LCMS, Perceived Stress Scale, Melatonin, Shiftwork, Nurse

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INTRODUCTION

There is currently a tendency for various jobs to take place for 24 hours, the changes daily rhythm of workers who have to work in shifts, especially at night. Various studies have shown high stress levels in workers (more than 20%, even 50%), especially in workers who have to work at night (Ginanti PD 2015, Techrisna E 2015, Marchelia V 2014, Irkhami *et al.*, 2015, Tantra S *et al.*, 2015, Maurits LS 2008). As is well known, chronic stress that continues for a period of time can cause disorders and illnesses, both physical and mental (Leka S *et al.*, 2010, Health and Safety Executive 2017). Therefore, it is necessary to do early detection for further prevention of stress cases. Unfortunately, an objective examination has not been used for early detection of workers experiencing stress, so the analysis is needed for workers who experience stress, especially workers who work shifts. This study examines melatonin levels in nurses who work shifts compared to nurses who work non-shifts.

METHODOLOGY

The study was conducted in a repeated cross-sectional study on shift and non-shift nurses who were randomly selected from December 2019 to June 2020 at a state hospital in Jakarta, Indonesia. For shift nurses, blood was collected at midnight on the last night shift or second night (pre-work), and 08.00 am (post-work) the next day, while for non-shift nurses at 08.00 am (pre-work) and at 04.00 pm (post-work) on the same day. Measurement of melatonin levels was conducted using Enzyme-Linked Immunosorbent Assay (ELISA) and Liquid Chromatography-Mass Spectrometry (LCMS). Before measuring melatonin levels, the stress level of shift and non-shift nurses was assessed subjectively using the Perceived Stress Scale (PSS) questionnaire. The inclusion criteria are shift and non-shift female nurses, aged 20-40, and have worked for at least one year. The exclusion criteria were workers with a history of mental emotional disorders, a history of metabolic diseases such as diabetes, heart disease, hypertension or problems with the menstrual cycle. This research has received permission from the Ethics Committee of the Faculty of Medicine, University of Indonesia number 867/UN2.F1/ETIK/PPM.00.02 / 2019 dated 29 July 2019.

RESULTS

The characteristics of the research subjects and the level of the PSS can be seen in *Table 1*.

Table 1: The Characteristics of Subjects and the Classification of Stress Level

Variable	Shift		Non Shift		Total	
	(n=40)		(n=40)		(n=80)	
	N	%	N	%	N	%
Age (years)						
31-40	9	11.3	23	28.7	32	40
20-30	31	38.8	17	21.3	48	60
Work experience (years)						
>/= 5	23	28.7	25	31.3	48	60
< 5	17	21.3	15	18.8	32	40
Marital status						
(+)	21	26.3	29	36.3	50	62.5
(-)	19	23.8	11	13.8	30	37.5
Stress level (PSS)						
severe	1	1.3	1	1.3	2	2.5
moderate	24	30	20	25	44	55
light	15	18.8	19	23.8	34	42.5

Table 1 shows that the non-shift nurses' group is mainly over 30 years old, with a length of work of more than or equal to 5 years and are already married. In contrast, the shift nurses are mostly younger, namely less or equal to 30 years old, with the same length of work and marital status as the non-shift nurse group. Overall, most of the respondents had worked for a long time, were married and were relatively young (20-30 years).

The PSS score results showed the moderate level of stress (score 14-26) in the Shift nurse is higher than the Non-Shift group. Only 2,5% in both groups showed a severe level of stress (score 27-40). This shows that the respondents' perceptions of stress from the shift nurses were higher than those of nurses who worked non-shift. This is following previous studies that showed high levels of stress in shift nurses (Terzieva DD *et al.*, 2009, Ahsan humaera hafid 2016, Pongoh V *et al.*, 2015).

Literature studies have not yet revealed a mean level of melatonin level at 12.00 pm midnight. Results from this study showed that mean level of Melatonin was 51.53 pg/mL (0.80-135.00 pg/mL) using ELISA and 52.35 pg/mL (10.00-170.00 pg/mL) by using LCMS in shift nurses, which was then compared to the level at 03.00 am according to the reference. Likewise, the value for mean melatonin levels at 04.00 pm is also unknown. The results of this study showed that the mean melatonin level was 10.84 pg/mL (2.15-38.30 pg/mL) using ELISA and 38.13 pg/mL (5.00-230,000 pg/mL) using LCMS in shift nurses. The complete data is presented in *Table 2*.

The description of the average melatonin levels can be seen in *Figures 1 and 2* below.

Figures 1 and 2 above show that melatonin levels measured using ELISA reveal a significant difference between the mean pre-work versus post-work, both in the shift group and in the non-shift group. It is also seen that the mean pre- and post- melatonin levels in the shift group are higher than the non-shift group. A different result was observed in the mean pre and post melatonin levels measurements using LCMS, where in the shift group melatonin levels was lower than the non-shift group.

Table 2: Melatonin levels in shift and non-shift nurses using ELISA and LCMS

Variable	NonShift (n=40) Pre :08.00 am Post :04.00 pm	%	N Shift (n=40) Pre :12.00 pm Post :08.00 am	%
Melatonin ELISA pre	(3,8-80,4pg/mL)	12.5	(18,5180pg/mL)	35.0
Smaller	5	2.5	14	0.0
Greater than	1	85.0	0	65.0
As per reference	34		26	
Average	17.1		51.5	
Median	13.0		45.6	
Min-Max	2.2-134.0 pg/mL		0.8-135.0 pg/mL	
Melatonin ELISA post	Reference (-)		(3,8-80,4 pg/mL)	0
below	-		0	2.5
	-		1	97.5
above	-		39	
As per reference	10.8		24.30	
Average	9.83		16.60	
Median	2.2-38.3 pg/mL		5.7-113.0 pg/mL	
Min-Max				

Melatonin LCMS pre	3.8-80.4 pg/mL	0.0	(18.5-180 pg/mL)	
below	0	37.5	7	17.5
above	15	62.5	0	0.0
As per reference	25		33	82.5
Average	361.8		52.4	
Median	15.0		50.0	
Min-Max			10.0-170.0 pg/mL	
Melatonin LCMS post	Reference (-)		(3.8-80.4 pg/mL)	
below	-		7	17.5
above	-		2	0.1
As per reference	-		31	77.5
Average	38.1		29.5	
Median	10.0		10.0	
Min-Max	5.0-230.0 pg/mL		10.0-190.0 pg/mL	

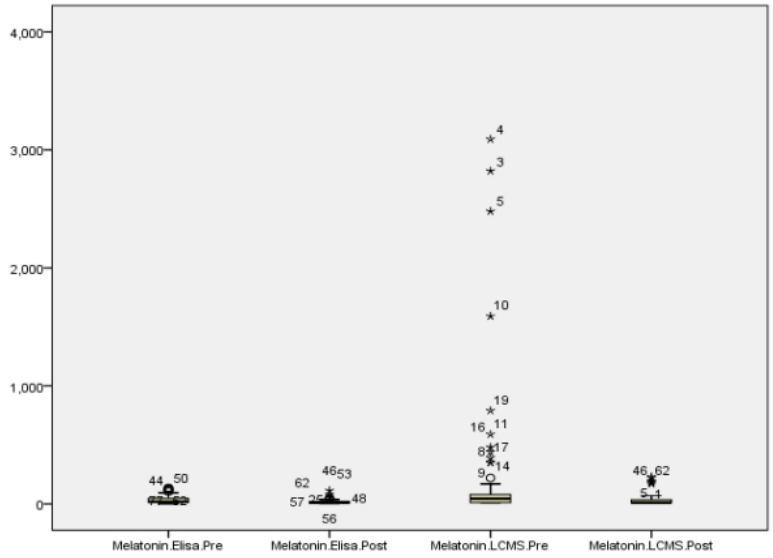
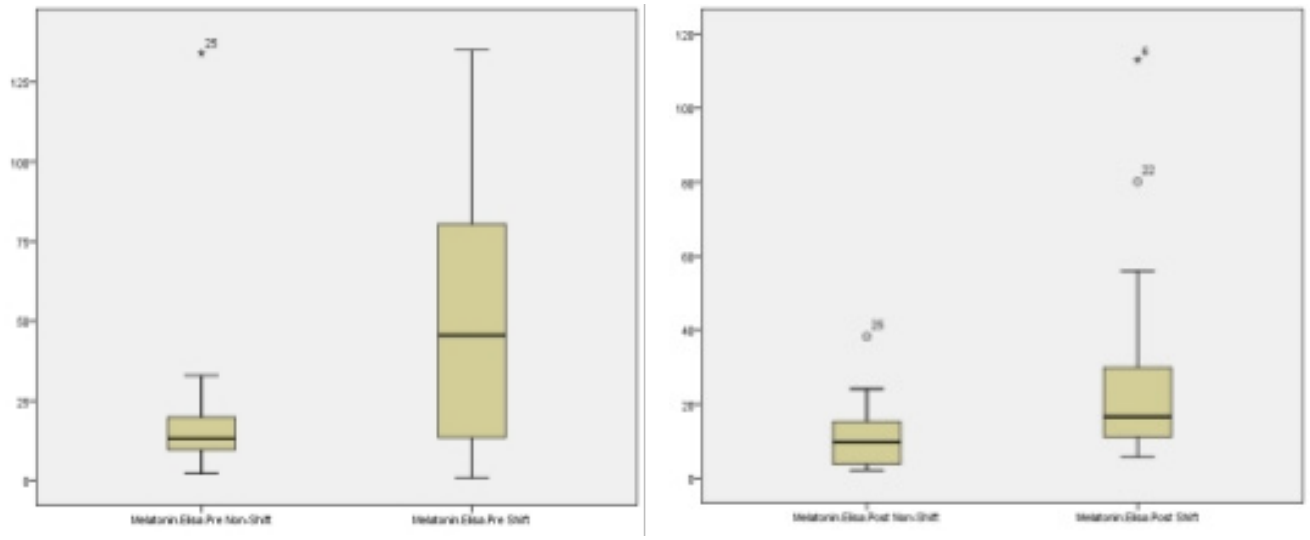


Figure 1: Average Pre and Post Melatonin Levels (ELISA) for All Respondents



(a) **(b)**

Figure 2a: Mean Melatonin ELISA Pre on non-shift & shift (mean 17.07 & 51.53)*
Figure 2b: Mean Melatonin ELISA Post on non-shift & shift (mean 10.84 & 24.30)*
*There was a significant difference between the shift and non-shift groups on melatonin levels (ELISA) during pre-work (Z = -3.873; p <0.001), and post-work(Z = -4.075; P<0.001)

DISCUSSION

Stress can cause sleep disturbances, such as insomnia, and reduced pineal peaks at night. Melatonin secretion often occurs during depression. A characteristic of changes in the rhythm of melatonin secretion has been observed in various mental disorders. 14-17 There are still only few studies that have looked at the presence of a melatonin profile in cases of stress. 17This study also showed that there was an inverse pattern in the dayshift group, namely that the average melatonin level tended to "decrease" in the afternoon compared to that in the morning. It is possible that at 08.00 am level of melatonin was still affected by the restful night before, while at 04.00 pm the melatonin level had not started to increase. Usually, melatonin levels in the afternoon or evening tend to be greater (Sherwood L 2014, Hall J 2014).

Measurement of melatonin levels using ELISA in the shift group showed a pattern in accordance with the previous literature, namely melatonin levels tend to decrease in the morning (mean 24.30 pg/mL) compared to the previous night (mean 51.53 pg/mL). This may occur due to the adaptation process of the shift group, which showed a pattern similar to normal conditions, even though they lack of sleep. Another study suggests that the pineal gland may be significantly affected by stress, which is consistent with findings that the pineal expresses a high density of glucocorticoid receptors. Melatonin receptors are also present in areas such as the hippocampus and adrenal glands (Musshoff U *et al.*, 2002, Torres-Farfan C *et al.*, 2003, Simonneaux V *et al.*, 2003). Recent data also showed that the rhythmic secretion of melatonin from the pineal gland is associated with modulating the release of neurotransmitters, especially serotonin and dopamine (Hemby SE *et al.*, 2003). It is also interesting to note that there were 35 percent (14 subjects) of shift respondents that showed a relatively low level of melatonin (below the mean level). However, in the morning it increased (change patterns) and became normal.

The study's results are similar with studies on rats, where the pineal gland expresses a high density of glucocorticoid receptors, suggesting the possibility of the gland being a target site for glucocorticoid breakdown during stress. It is similar to other areas, such as the hippocampus, which is highly sensitive to stress and prolonged glucocorticoid secretion during chronic stress, and may have a damaging effect on the pineal gland (Morera AL *et al.*, 2006). Prolonged secretion of glucocorticoids during chronic stress can cause deleterious effects in the pineal in terms of inhibition of glucose transport, and a more rapid decrease in ATP concentration and metabolism in pinealocytes, similar to those proposed in hippocampal stress damage. Chronic stress can interfere with pineal sympathetic innervation as well as rhythmic secretion of melatonin, which may affect the information transmitted to more brain areas, because the hippocampus has high levels of melatonin receptors and regulates limbic-HPA as well as the sympathetic adrenergic-noradrenergic systems. Collectively, these findings suggest that melatonin may be significantly related to memory regulation, cognition, and also to be involved in emotional processing (Musshoff U *et al.*, 2002, Torres-Farfan C *et al.*, 2003, Simonneaux V *et al.*, 2003).

It is well knows that measurements using the LCMS method quantity melatonin core compounds and there is no cross reactivity between melatonin and other compounds, as in the ELISA measurement. In measuring melatonin levels with ELISA, cross reactivity can occur with 5-Methoxy-Triptophole (1.2%), N-Acetyl Serotonin (1.2%) and 5-Methoxy Tryptamine (2.5%). Compared to the results of measurements using LCMS, the mean pre- and post-melatonin levels in the shift group were smaller than the non-shift group. The LCMS results (both in the shift and non-shift groups) showed a pattern of melatonin levels which is according to the pattern described in the previous literature, showing a tendency to be lower in the morning compared to at night. There was no correlation between melatonin levels in this study, either using ELISA or using LCMS, and PSS scores for shift and non-shift nurses.

CONCLUSION

This study showed differences in the results of measuring melatonin levels using ELISA and LCMS, which will be a good starting point to reveal in future studies with a larger number of respondents. In addition, it is interesting to see that in both the shift and non-shift groups, a decrease in the mean level of melatonin (measured using ELISA and LCMS) from pre-work to post-work was observed.

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