Measuring of Plasma Melatonin Level in Patients with Preeclampsia

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Abstract:

Background: disturbed physiological rhythm of blood pressure in preeclampsia is a common finding. The role of oxidative stress in pathogenesis of preeclampsia is well accepted. Melatonin is a powerful free radical scavenger so it's rapidly consumed by enhanced reactive oxygen species in preeclampsia causing non-dipping in blood pressure.

Objective: To evaluate the change in plasma melatonin levels in patients with preeclampsia and its relationship with blood pressure.

Patients and methods: In this prospective case control study a total of 40 primigravidae pregnant women were recruited during the period of 11 months between August 2015 and August 2016 in Baghdad teaching hospital, medical city, Iraq, divided into two groups:

First groups: (cases group) were 20 primigravidae pregnant women with PE.

Second group: (control group) were 20 normal healthy primigravidae.

Blood Pressure measurement, melatonin blood samples were taken, plasma melatonin levels measurement was done by ELISA immunoassay. Urine was collected over 24 hours for protein in urine measurement.

Results : Plasma Melatonin level in control , day and night was (22.72 ± 2.6 pg/mL) , (75.26 ± 2.99 pg/mL) compared to Plasma Melatonin level in dipper PE day and night (20.5±2.4 pg/mL) , (75.26 ± 1.8 pg/mL) which was statistically not significant(P value 0.055) , (P value 1.0) respectively .

Plasma Melatonin level for non-dipper (22.45 ± 2.48 pg/mL) were similar to dipper (20.5±2.4 pg/mL) which is not significant (P value 0.1) , while Night time Plasma Melatonin of non-dipper (36.76 ± 1.27) were reduced when compared to control (75.26 ± 2.99 pg/mL) and to dipper group (75.26 ± 1.8 pg/mL) which was highly significant ($p < 0.0001$, $p < 0.0001$) respectively .

Conclusion: Night time Plasma Melatonin level reduced in Primigravid Women with preeclampsia that did not show nocturnal dipping in blood pressure.

Keywords: Melatonin hormone, melatonin during pregnancy, melatonin levels in preeclampsia, preeclampsia.

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Introduction:

Pre-eclampsia or preeclampsia (PE) is a disorder of pregnancy characterized by high blood pressure and a large amount of protein in the urine.(1) with Significant maternal and fetal morbidity and death. It can progress to an even more serious condition, eclampsia, which is accompanied by life threatening seizures (2). Affecting 10% of all pregnancies worldwide (3).As the condition progresses, the damaged placenta releases molecules into the circulation which cause endothelial dysfunction leading to an elevation in blood pressure and eventually more systemic damage (4) Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous lipid-soluble antioxidant hormone produced primarily by the pineal gland in humans, providing circadian and seasonal timing cues, It is powerful antioxidant, acting both as a direct scavenger of oxygen free radicals, especially the highly damaging hydroxyl radical, and indirectly via up-regulation of antioxidant enzymes including GSHPx, GSH-reductase, superoxide dismutase and *Hospital*catalase(5)**.** It's main physiological functions are related paracrine properties(6) Of melatonin secretion is thought to contribute to other functions of the circadian clock(7,8).Melatonin displays high lipid and water solubility, which facilitates passage across cell membranes.(6)After release in the circulation, it gains access to various fluids, tissues , as no pineal storage of melatonin is available, the plasma hormone profile faithfully reflects the pineal activity(9)the secretion occurs at night, with maximum plasma levels around 03:00–04:00 whereas diurnal levels are undetectable, or low in rested subjects (6) Melatonin function : Antioxidant (10), Metal chelation**(11),** Immunomodulation**(12),** in assisted reproductive technology**(5),** Melatonin use in Cardiovascular disease(13)**,** It's role in pregnancyhas also been shown to up-regulate antioxidant enzymes in human pregnancies leading to the conclusion that melatonin might provide an indirect protection against injury caused by reactive oxygen specieses seen in preeclampsia, and fetal hypoxia(14)**,** Melatonin levels increases throughout pregnancy, reaching a peak at term.(15)Melatonin is also found in amniotic fluid and its levels positively correlate with those of maternal blood.(16)It's easily and rapidly crosses the placental

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barrier to enter the fetal circulation (17)and is implicated in placental functions and fetal development in both human and animal models. It specifically entertains the circadian rhythms and promotes fetal growth and neurogenesis.(18)and regulate placental hormone production**(19)**, so placental melatonin levels may be altered in pathological pregnancies(20). Preeclampsia is a condition of elevated oxidative stress with high free radical generation and reduced antioxidants (21). Given the antioxidant properties of melatonin, its use may be helpful in reducing the systemic oxidative stress associated with preeclampsia (22). Blood pressure (BP) in healthy individuals follows a characteristic circadian pattern with a nocturnal decline of 10–20% followed by an increase early in the morning. (23) In some patients with hypertension or chronic kidney disease however, BP fails to dip during night and these individuals have been called ‘‘non-dippers’’, in contrast with those with a normal nocturnal dip, who are called ‘‘dippers’’. Melatonin influences rhythmic changes in BP by affecting the master clock localized in the suprachiasmatic nucleus of the hypothalamus, so present study aimed to correlate Melatonin level and its use as marker for severity of preeclampsia. (24, 25)

Patients and methods:

A prospective case-control study conducted at Baghdad teaching hospital from August 2015 to August 2016 in which 40 women were included in present study and divided into two groups.

First groups : (cases group) were 20 primigravid women with preeclampsia (PE), further divided in to two subgroups , dipper PE with a normal nocturnal dip of blood pressure , non-dipper PE patient whose blood pressure failed to dip during night . Second group : (control group) were 20 normal healthy primigravidae.

Inclusion criteria: Have a diagnosis of preeclampsia **,** age of at least of 18 years old **,** gestational age of 32 weeks and more **,** primigravidity , a singleton viable pregnancy , no major congenital fetal anomalies evident on ultrasound scan **,** be capable of understanding the information provided**.**

Exclusion criteria: Chronic hypertension **,** Eclampsia , Multiparty , Multiple pregnancy , Diabetes , Chronic liver, renal, or heart disease , Cancer and depression , Intra uterine death . The demographic characteristics of each patient were assessed including maternal age , gestational age examination , Blood pressure was measured by mercury sphygomomanometer in left lateral position taken for each women at day time 12pm and another reading at night time 12am (at night reading the women instructed to go to bed at 9.00pm with light of the room switched off). In mild PE blood pressure equal to or more than 140/90 mmHg in two consecutive readings four hours apart with proteinurea >0.3g in 24-hours urine collection in more than 20 weeks of gestation , PE considered as sever if diastolic blood pressure >110 mmHg or sever protein urine >5g in 24 hours urine collection. Plasma melatonin samples were measured in both groups (cases &controls) in the middle of the dark and light phase at 12.00am and 12.00pm hours usually at time of BP measurement. Blood samples were withdrawn from antecubital vein of 2.5 ml for each sample, after centrifugation for 10 minutes then the blood plasma placed in EDTA tube and frozen at -20 °C to -80 °C until the measurement of plasma melatonin level. Plasma melatonin level was measured by enzyme-linked immunoassay (Human melatonin, MT ELISA Kit, CUSABIO BIOTECH) the minimum detectable dose of plasma MT is typically less than 1.56 pg/ml,and intra assay < 8%, and inter-assay < 10%. The detection range of the MLT kit is 6.25pg/ml – 400pg/ml. Mean arterial pressure (MAP) was measured using the standard formula: MAP=Diastolic BP+1/3(Systolic BP-Diastolic BP). A nocturnal dipping in MAP for more than 10-20% mmHg in both groups was considered as a nocturnal dippers women as a nocturnal response to midnight increase in plasma melatonin secretion and women of nocturnal dipping in MAP of less than 10% mmHg was considered as nocturnal non- dippers women .

Statistical Analysis: The data of total 40 women were collected and analyzed using the statistical package for social sciences (SPSS) Windows version21-2016. The Values were expressed as a mean, standard deviation (mean±SD), numbers and proportion (%). Inter-group differences were compared by using student t- test, and the p –value of less than <0.05 considered statistically significant and p-value of less than 0.001 considered as highly statistically significant. The findings and results were presented in figures and tables with an explanatory paragraphs.

Results:

The current study included 40 primigravidae pregnant women, whose gestational age ranges from 32 – 40 weeks, and their age ranges from 20 – 30 years old **,** Most data were expressed by (mean ± standard deviation). As expected the daytime and night time MAP of PE group with mean value of (123.35±6.75, 117.66±9.9)respectively, and the daytime, night time of controls group with mean value of (98.64±6.24,86.26±5.38) respectively, were significantly higher in PE groups than controls group with p <0.001,this finding is shown in table 1.

Table 1: comparison of daytime and nighttime MAP, PMT between normal pregnant women and women with PE

FACTORS	NP (N=20)	PE (N=20)	P VALUE
Daytime MAP (mmHg)	98.6 ± 6.24	123.35 ± 6.75	< 0.001
Nighttime MAP (mmHg)	86.26 ± 5.38	117.66 ± 9.9	< 0.001
Daytime PMT (pg/mL)	22.75 ±2.6	23.61 ± 3.64	0.391
Nighttime PMT (pg/mL)	75.26 ± 2.99	50.61 ± 18.15	< 0.001

MAP did not reach the statistical difference in day and night times in PE women,mean values (123.35±6.75, 117.66±3.64 mmHg) p value=0.4627, confidence interval =5.69 mmHg

That means MAP dipping less than 10% mmHg in PE women

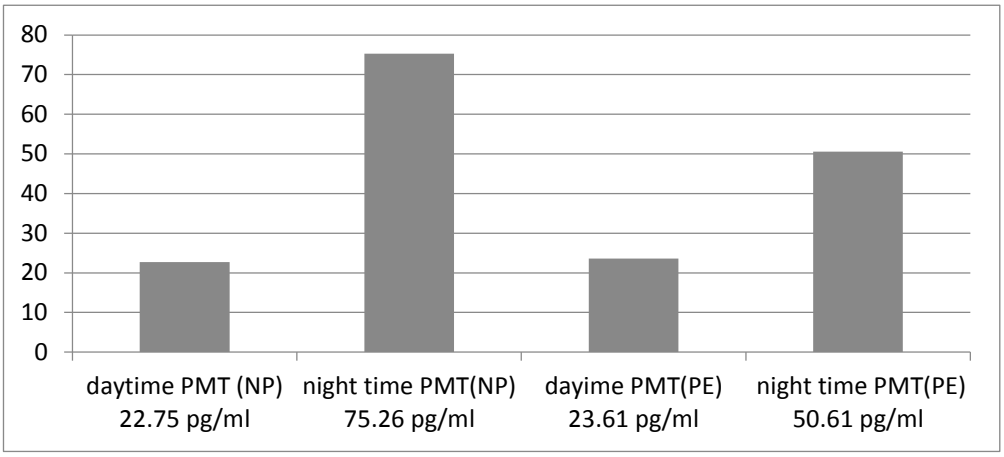

Figure 1: daytime and nighttime PMT levels in normal pregnant women and women with preeclampsia .

The inter-group comparison between nocturnal non-dipping and dipping groups with preeclampsia shown in table 2, that contains all variable factors in the PE women regarding their maternal age (years), GA (weeks) and proteinuria (mg/dL) and BMI (kg/m²), all were observed higher in non-dipping women but they do not reach the statistical significance as shown in table 2. Severe PE was noticed in 5 cases out of 13 women in non-dippers group (38%) and no cases of severe PE were found in dippers group. This clearly implicate that non-dippers group in present study includes cases with severe PE (table 2).

Table 2: inter-group comparison between dippers and non-dippers groups of PE women regarding their age, GA, BMI, severe PE and proteinuria.

Variables	Dippers group(n=7)	Non-dippers group (n=13)	P value
Age (years)	24.2 ± 6.3	25.8 ± 4.5	0.495
GA(weeks)	36.2 ± 3.2	37.3 ± 2.6	0.415
BMI (kg/m²)	29.5 ± 3.5	32 ± 2.1	0.0594
Severe PE {n(%)}	None	5 (38%)	None
Proteinuria (mg/dL)	930 ± 550	2100± 1871	0.1271

As presented in table (2) there was no significant difference in daytime MAP between dippers group and non-dippers group (p=0.1138), but there was significant difference in nighttime MAP (p=<0.0001), that means the circadian BP rhythm physiological in all dippers group (n=7), and disrupted circadian rhythm in all non-dippers group

Table 3: showing the comparison of daytime and night time MAP,daytime and night time PMT levels between dippers and non-dippers groups with preeclampsia .

Variables	Dippers group (n=7)	Non-dippers group (n=13)	P value
Daytime MAP (mmHg)	120 ± 6.2	125.16 ± 6.79	0.1138
Night time MAP (mmHg)	106.8 ± 5.5	123.4 ± 6.6	< 0.0001
Daytime PMT (pg/mL)	20.5 ± 2.4	22.45 ± 2.48	0.1073
Night time PMT(pg/mL)	75.26 ± 1.8	37.62 ± 1.26	<0.0001

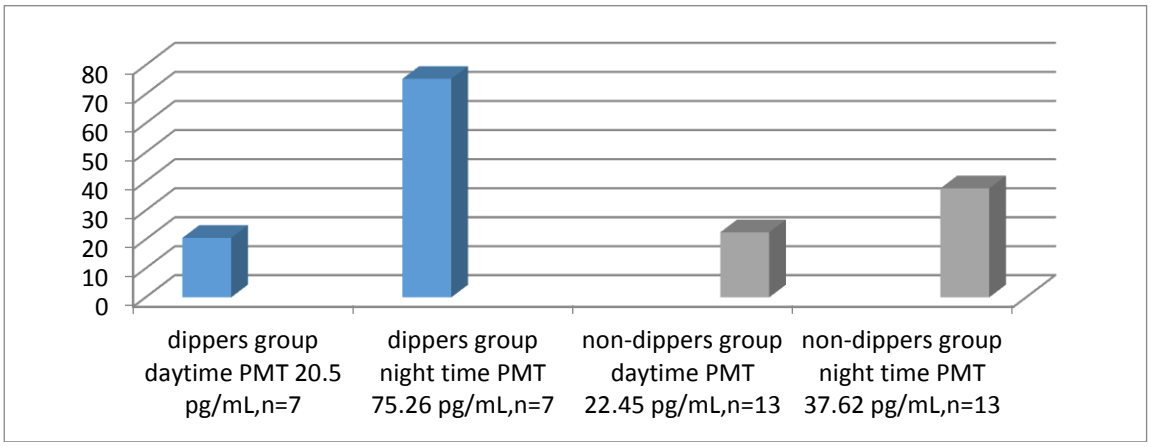

Figure 2: comparison between daytime and nighttime plasma melatonin levels in PE women and Normal healthy women.

The day-night rhythm of plasma melatonin secretion was disturbed in the group of non-dippers with PE and their nighttime plasma melatonin levels were significantly lower compared to dippers group (37.62±1.26 vs. 75.26±1.8pg/mL, p<0.0001). In contrast, daytime plasma melatonin levels in non-dippers were similar to that in dippers group(22.45±2.48 vs. 20.5±2.4, p=0.1073) as shown in table 3 **.**

Discussion:

In the current study we investigated the plasma melatonin levels at daytime and nighttime in(40) women, (20) of normal heathy pregnancy and their circadian rhythm of BP affected by plasma melatonin level . The result in the current study showed that all (20)healthy pregnant women have the physiological rhythm of BP and normal levels of plasma melatonin secretion at daytime and nighttime measurement , while 20 women with preeclampsia were of two groups , the dipping group were have the normal physiological rhythm of BP and melatonin secretion of daytime and nighttime melatonin secretion , the second or non-dipping group the BP did not show the normal nocturnal dipping and nocturnal melatonin secretion was decreased of daytime and nighttime compared to normal healthy pregnant women and dipping group with PE . This is concordant with that revealed by Bouchlariotou et al2014(23) whom study included 51 women that measuring serum and urine melatonin levels ,20 women of normal healthy pregnancy all were dipper with normal melatonin secretion and 31 women with preeclampsia, women with preeclampsia (68%) non-dipper with reduced nighttime melatonin secretion (P value< 0.001) compared to normal healthy pregnant women , and (32%) were dippers with normal rhythm of BP and daytime , nighttime melatonin secretion. Also concordant with that revealed by Nakamura et al 2001 (23),study showed serum melatonin secretion in preeclampsia significantly lower than serum melatonin secretion of normal pregnant women after 32 weeks of gestation . Also concordant with that revealed by Zeng et al2016(26), serum was collected from women with preeclampsia the serum levels of melatonin were significantly reduced in women with preeclampsia at presentation , while its inconsistent with Tranquilly et al 2004 (27),study of 16 primigravidae showed the rhythm of melatonin secretion was lost in all pregnant women with lost of blood pressure rhythm and preeclamptic women have higher melatonin concentrations (P value<0.001) , this may be due to small sample size and in present study we carefully selected patient ages (20-30) because melatonin secretion is age-related secretion that higher levels in children and the lower levels in elderlies and to avoid age-related differences we selected age ranges between (20 -30) years old . Regarding the severe preeclampsia as indicated by high MAP or proteinuria, we found no significant statistical difference in daytime plasma melatonin levels (p value0.7856), and nighttime plasma melatonin levels (p value0.2901) , so no relationship between the severity of preeclampsia and reduced nighttime plasma melatonin levels , which agree with Bouchlariotou et al. 2014 (23) who also found no relationship between severe and non-severe cases of preeclampsia and melatonin levels (p value 0.106) . This is concordant with Zeng et al (2016)(26) ,who showed that reduced levels of melatonin in patients with preeclampsia not associated with the severity of preeclampsia . But it's not concordant with Nakamura et al 2001(20), whom results showed that patients with severe preeclampsia have significantly lower serum melatonin levels than mild preeclampsia or normal healthy pregnant women after (32) weeks of gestation, this is may be attributed to different timing of measurement during pregnancy and in their study (second) while our study done in the third trimester only. The limitations in this study are no (24) hours continuous BP monitoring used to evaluate BP changes which gives more accurate results instead we used twice BP measurement, one at daytime and one at nighttime melatonin levels were only measured in blood plasma and melatonin level in urine did not measured and compared which also gives more accurate results.

Conclusion:

Night time Plasma Melatonin level reduced in Primigravid Women with preeclampsia who did not show nocturnal dipping in blood pressure.

Authors Contribution:

Maad M. Shalal: supervisor
Ishraq M. Kadhim: sample collector and workup.
Nada Saed Abbas: sample collector and workup.
GhaidaaAbdulsattar: student.

References:

1. Al-Jameil N, Aziz Khan F,Fareed Khan M, Tabassum H (2014) A brief overview of preeclampsia. Jclin med res; 6(1):1–7.

2. Reiter1 RJ, TanDX, KorkmazA,Rosales-CorralSA(2014) Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology. Hum Reprod Update;20(2):293–307.

3. WHO (2011) WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. Geneva: World Health Organization; PP. 1-48. Available at http://whqlibdoc.who.int/publications/2011/9789241548335_eng.pdf (accessed 9 December 2014).

4. Roberts JM, Hubel CA (2009)The two stage model of preeclampsia: variations on the theme. Placenta;30(Suppl A):S32-7.

5. HobsonSR,Lim R, Gardiner EE, Alers NO, WallaceEM(2013) Phase I pilot clinical trial of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by pre-eclampsia (PAMPR): study protocol. BMJ Open; 3(9):e003788.

6. Claustrat B, Brun.J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. Sleep Med Rev; 9(1):11–24.

7. Dijk DJ, Cajochen C (1997) Melatonin and the circadian regulation of sleep initiation, consolidation, structure, and the sleep EEG. J Biol Rhythms; 12(6):627–35.

8. Krauchi K, Wirz-Justice A (2001) Circadian clues to sleep onset mechanisms. Neuropsychopharmacology; 25(5 Suppl.):S92–S96.
9. Reiter RJ (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev; 12(2):151–80.
10. Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F (1994) Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci. 55 (15): PL271–6.
11. Limson J, Nyokong T, Daya S (1998) The interaction of melatonin and its precursors with aluminium, cadmium, copper, iron, lead, and zinc: an adsorptive voltammetric study. J Pineal Res; 24(1):15–21.
12. Pohanka M (2013) Impact of melatonin on immunity: a review. Central Eur J Med; 8(4):369–76.
13. Wakatsuki A, Okatani Y, Ikenoue N, Kaneda C, Fukaya T (2001) Effects of short-term melatonin administrationon lipoprotein metabolism in normolipidemic postmenopausal women. Maturitas; 38:171-7.
14. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y (1998) Maternal-fetal transfer of melatonin in pregnant women near term. J pineal res.; 25(3):129-34
15. Kivela A (1991) Serum melatonin during human pregnancy. ActaEndocrinol; 124(3):233–237.
16. Kivela A, Kauppila A, Leppaluoto J, Vakkuri O (1989) Serum and amniotic fluid melatonin during human labor. J ClinEndocrinolMetab; 69(5):1065–8.
17. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y (1998) Maternal-fetal transfer of melatonin in pregnant women near term. J Pineal Res; 25(3):129–34.
18. Seron-Ferre M, Mendez N, Abarzua-Catalan L , Vilches N, Valenzuela FJ, Reynolds HE, Llanos AJ, Rojas A, Valenzuela GJ, Torres-Farfan C (2012) Circadian rhythms in the fetus. Mol Cell Endocrinol; 349(1):68–75.
19. Iwasaki S, Nakazawa K, Sakai J, Kometani K, Iwashita M, Yoshimura Y, Maruyama T (2005) Melatonin as a local regulator of human placental function. J Pineal Res; 39(3):261–5.
20. Nakamura Y, Tamura H, Kashida S, Takayama H, Yamagata Y, Karube A, Sugino N, Kato H (2001) Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. J Pineal Res; 30(1):29–33
21. Reiter RI, Tan DX, Manchester LC, Paredes SD, Mayo JC, Sainz RM (2009) Melatonin and Reproduction Revisited. BiolReproduc; 81(3):445–56.
22. Milczarek R, Klimek J, Zelewski L (2000) Melatonin inhibits NADPHdependent lipid peroxidation in human placental mitochondria. HormMetab Res; 38(2):84–85.
23. Bouchlariotou S, LiakopoulosV, Giannopoulou M, Arampatzis SP, EleftheriadisT,Mertens PR, Zintzaras E, MessinisIE,StefanidisI (2014) Melatonin secretion is impaired in women with preeclampsia and an abnormal circadian blood pressure rhythm.Ren Fail; 36(7):1001-7.
24. O'Brien E, Sheridan J, O'Malley K (1988) Dippers and non-dippers. Lancet;2(8607):397.
25. White WB (1999) Ambulatory blood pressure as a predictor of target organ disease and outcome in the hypertensive patient. Blood Press Monit;4(3-4):181–4.
26. Zeng K. Gao Y, Wan J, Tong M, Lee AC, Zhao M, Chen Q (2016) the reduction in circulating levels of melatonin may be associated with the development of preeclampsia. J Hum Hypertens;30(11):666-671.
27. Tranquilli AL ,Turi A, Giannubilo SR, Garbati E (2004) Circadian melatonin concentration rhythm is lost in pregnant women with alterd blood pressure rhythm. GynecolEndocrinol;18(3);124-9.