A CORRELATION BETWEEN THE FLUCTUATIONS OF CYTOKINE CONCENTRATIONS MEASURED IN THE MORNING AND EVENING AND THE CIRCADIAN BLOOD PRESSURE RHYTHM IN PATIENTS WITH STAGE II ESSENTIAL HYPERTENSION

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Today, increasing attention is being paid to the role of circadian rhythms in pathology. There are time-of-day-dependent immune markers that provide valuable information about disease progression. The aim of this study was to measure evening and morning concentrations of a few cytokines (interleukins, adhesion molecules, tumor necrosis/growth factors, etc.) in the peripheral blood of patients with stage II essential hypertension and to investigate how they correlate with a nocturnal blood pressure decline. Blood samples were collected from 90 patients with stage II EH at 7:00 a.m. and 8:00 p.m. Cytokine concentrations were measured using immunoassays. Based on 24-h blood pressure monitoring, the patients were distributed into 3 groups: dippers, non-dippers and night-peakers. The morning to evening ratios of cytokine concentrations in patients with EH differed from those in healthy controls due to an increase in the evening concentrations of somnogenic cytokines (IL1β, IL1α) and LIF, sLIFr, and M-CSF whose daily fluctuations patterns remain understudied. On the whole, the fluctuation patterns of the measured cytokines in patients with stage II EH who had had the condition for 10 to 14 years and were receiving no antihypertensive treatment at the time of our study differed from those displayed by healthy controls. A twenty percent rise in the evening concentrations of IL1α, LIF, sLIFr, M-CSF, and erythropoietin contributes significantly to pathological blood pressure rhythms (as demonstrated by the groups of non-dippers and night-peakers) in patients with stage II EH receiving no antihypertensive therapy. Understanding the pathophysiological role of cytokine levels and their fluctuations over a 24-h cycle could inspire new methods for EH prevention and reduce end-organ damage.

Keywords: cytokines, essential hypertension, Dipper, Non-dipper, Night-peaker

СВЯЗЬ СУТОЧНЫХ КОЛЕБАНИЙ СОДЕРЖАНИЯ ЦИТОКИНОВ С ИЗМЕНЕНИЕМ РИТМОВ АРТЕРИАЛЬНОГО ДАВЛЕНИЯ ПРИ ЭССЕНЦИАЛЬНОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ ВТОРОЙ СТАДИИ

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Все больше внимания уделяется исследованию роли суточных ритмов в патологических процессах в организме. Безусловно, существуют суточные иммунные маркеры, позволяющие судить о прогрессировании ряда заболеваний. Целью исследования было изучить содержание цитокинов (интерлейкинов, молекул адгезии, факторов некроза опухоли, роста и др.) в сыворотке периферической крови больных эссенциальной артериальной гипертензией (ЭАГ) II стадии в утреннее/вечернее время и его корреляцию со степенью снижения артериального давления (АД) в ночное время. У 90 пациентов с ЭАГ II стадии проводили забор крови в 7.00 и 20.00 ч, определяли иммуноферментным методом в сыворотке периферической крови цитокины и проводили анализ данных суточного мониторирования АД с выделением групп: «Dipper», «Non-dipper», «Night-peaker». Обнаружено изменение отношения утренних и вечерних концентраций при сравнении со здоровыми, за счет повышения степени роста в вечернее время как цитокинов из группы «somnogenic» (IL1β, IL1α), так и мало изученных в аспекте суточных закономерностей LIF, sLIFr, M-CSF. Динамика и уровни исследуемых показателей у пациентов с ЭАГ II стадией и анамнезом заболевания 10–14 лет без гипотензивной терапии отличались от таковых в группе здоровых добровольцев (контроля). Повышение концентраций IL1α, LIF, sLIFr, M-CSF, эритропоэтина в 20.00 ч на 20% и более является значимым компонентом формирования патологических околосуточных ритмов АД («Non-dipper» и «Night-peaker») у больных ЭАГ II стадии при длительности заболевания 10–14 лет (без приема гипотензивных препаратов). Понимание патофизиологической роли изменения не только количественных характеристик цитокинов сыворотки периферической крови у больных ЭАГ II стадии, но и особенностей их суточной динамики может стать основой создания новых систем профилактики прогрессирования ЭАГ и снизить частоту повреждения органов-мишеней.

Ключевые слова: цитокины, эссенциальная артериальная гипертензия, «Dipper», «Non- dipper», «Night-peaker»

Circadian rhythms are adaptive oscillations in behavior and physiology that follow a roughly 24-h cycle and prepare an organism for the environmental effects of the Earth's rotation [1]. The circadian clock originated in cyanobacteria and is found in all multicellular organisms and mammals in particular. In 2017, the Nobel prize in physiology or medicine was awarded to Jeffrey C. Hall, Michael Rosbash and Michael W. Young "for their discoveries of molecular mechanisms controlling the circadian rhythm". This award has emphasized the importance of research into the role of daily cycles in health and pathology. In 1960 Halberg et al. articulated the basic principles of circadian rhythm research in immunology after discovering a circadian pattern of sensitivity to an endotoxin administered to mice at different times of day. The sleep/wake cycle is one of the most well-studied manifestations of the circadian rhythm, correlating with the immune response and blood pressure (BP) dynamics [2]. It is currently assumed that proinflammatory cytokines are somnogenic, i.e. increasingly synthesized during the resting phase, whereas anti-inflammatory cytokines, such as IL10, are activated upon awakening and inhibit sleep. In this respect, IL1β, TNFα, IFNγ, IL6, IL4, and IL10 are currently the best studied [1, 3, 4, 5]. Blood pressure control is exerted by the following neuroendocrine mechanisms: the monoaminergic systems that regulate the physiological activity of the autonomous nervous system and the secretion of biogenic amines; the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes; the opioid, renin-angiotensin-aldosterone and endothelial systems, and vasoactive peptides [6, 7]. Recently, there has been growing interest in circadian rhythms and their effect on the immune system, but in spite of significant advances in the understanding of their mechanisms, many circadian phenotypes are yet to be explained. A number of experimental studies have suggested an association between the fluctuations of cytokine levels at different times of day and end-organ damage [8] and also a nocturnal decline in BP [9]. Progredient essential hypertension (EH) accompanied by stably high BP with very slight fluctuations in the evening correlates with the levels of certain cytokines. This suggests the existence of time-of-day-dependent immune markers that promote cardiovascular diseases and provides rationale for our study. This work aims to investigate the association between the morning/evening levels of certain cytokines (interleukins, adhesion molecules, tumor necrosis/growth factors, etc.), as well as erythropoietin, in the peripheral blood of patients with stage II EH and the nocturnal BP decline registered in such patients.

METHODS

The study was carried out at the facilities of the Regional Vascular Center of Mordovian Republican Clinical Hospital No.4, Mordovian Republican Clinical Hospital No.3 and the Department of Immunology, Microbiology, and Virology of Ogariov Mordovian State University. The study recruited 90 patients (40 men and 50 women). Fifty healthy volunteers with no signs of EH constituted the control group. The groups were comparable in terms of age and sex. The following inclusion criteria were applied: age of 57.5 ± 1.17 years; stage II EH (duration of 10 to 14 years); no antihypertensive treatment received. Exclusion criteria included comorbidities; types I and II diabetes mellitus; metabolic syndrome; autoimmune disorders; allergies; symptomatic therapy aimed to normalize blood pressure; infections 1 month before the study; alcoholism or drug addiction; refusal to participate in the study. Peripheral blood samples were collected from the patients and healthy controls at 7:00 a.m. and 20:00 p.m. The interval between blood collection and meals was at least 6 h. The following cytokines were assayed: IL1β, IL1α, IL1ra, IL18, IL18BP, IL37, IL6, sIL6r, LIF, sLIFr, TNFα, sTNF-RI, IL2, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, M-CSF, erythropoietin, and a few vasoactive peptides, including NO, iNOS, eNOS, ADMA, SDMA, Nt-proCNP, and Nt-proBNP. In 20% of patients, blood sampling was repeated a month later to test the reproducibility of the results. The variance was 3 to 6%. Time for blood collection was chosen based on the literature [1, 8, 10] and our own pilot study which we had conducted in 5 healthy individuals and 24 patients with stage II EH with different circadian BP rhythms. In that study, we had analyzed the levels of 6 cytokines, including IL1α, LIF, sLIFr, M-CSF, and erythropoietin, measured at 6:00 a.m., 8:00 a.m., 12:00 a.m., 4:00 p.m., 8:00, and midnight.

Peripheral blood concentrations of the studied cytokines and vasoactive peptides were determined with ELISA using the Personal Lab TM analyzer (Adaltis; Italy) at the laboratory of the Department of Immunology (Ogariov Mordovian State University; laboratory license 13.01.04. 0001.L.000005.06.11 issued 23.06.2011).

All study participants had their blood pressure monitored at the Regional Vascular Center of the Mordovian Republican Clinical Hospital 4 and Mordovian Republican Clinical Hospital 3 using Space Labs Medical 90702 (Spacelabs Medical, Inc.; USA) according to the recommendations of the Fourth International Consensus Conference on Ambulatory Blood Pressure Monitoring (Belgium, 1994); the obtained blood pressure values were added to the patients' medical histories. The patients were divided into 3 groups depending on the degree of BP fluctuations at night: dippers (10–20% decline), non-dippers (< 10% decline); night-peakers (elevated BP).

The data were processed in Statistica ver. 8.0. (StatSoft Inc.; USA). Normality of data distribution was tested using the one-sample Kolmogorov-Smirnoff test. If the distribution was normal, the data were presented as an arithmetic mean (M) and a standard deviation (SD); if the distribution deviated from the norm, the data were presented as a median (Me) and the interquartile range (C25–C75). Depending on the normality of data distribution and the sample size, the two-sample t-test and the Mann-Whitney U-test were used to compare unrelated samples; related samples were analyzed using the paired t-test and Wilcoxon signed rank test.

RESULTS

The patients with stage II EH who had been suffering from this condition for 10 to 14 years and were not receiving antihypertensive therapy around the time of the study, had higher morning concentrations of IL1β, IL1α, IL18, IL18BP, IL6, sIL6r, LIF, TNFα, sTNF-RI, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, M-CSF, and erythropoietin ($p < 0.001$) and lower morning concentrations of IL1ra ($p < 0.001$) than the healthy controls (Table 1). The following dynamics were observed in the control group in the evening: IL1β levels rose by 14.1% (0.37), $p < 0.05$; IL1α, by 14.8% (0.33), $p < 0.05$; IL6, by 29.2% (0.98), $p < 0.001$; TNFα, by 12.3% (0.78), $p < 0.01$; sTNF-RI, by 12.6% (0.65), $p < 0.05$; IL1ra dropped by 14.6% (0.32), $p < 0.01$; IL10, by 23% (0.58), $p < 0.01$, and sIL6r, by 30.4% (1.54), $p < 0.001$. In the patients with stage II EH the pattern was the same as in the controls, but the changes were more pronounced: IL1β concentrations rose by 21.3% (0.92), $p < 0.001$ and IL1α, by 31.2% (1.12), $p < 0.001$. The most significant, clinically relevant differences registered in the group of patients with EH were observed for LIF concentrations that increased by 16% (0.43),

$p < 0.01$, and for sLIFr that demonstrated a 22% rise (0.63), $p < 0.001$. In our hypertensive patients, M-CSF levels increased by 20.2% (0.42), $p < 0.001$, and erythropoietin, by 36.5% (1.22), $p < 0.001$, whereas IL18BP concentrations declined by 11% (0.27), $p < 0.05$ and IL37, by 21.7% (0.86), $p < 0.001$, in contrast to the control group. The evening rise in the IL6 and TNFα concentrations was comparable between the two groups: IL6 levels surged by 31% (1.02) and 29.2% (0.98), $p < 0.001$, respectively, while TNFα levels rose by 12.3% (0.78) and 12.2% (0.66), $p < 0.01$), respectively. In the healthy volunteers, sIL6r levels decreased in the evening ($p < 0.001$), attenuating IL6 activity after 8 p.m. In contrast, the patients with elevated blood pressure did not have this protective sIL6r decline. The analysis of evening IL10 concentrations revealed a comparable decline ($p < 0.01$) in the levels of this proinflammatory cytokine between the groups, which reached 17% (0.58) and 15% (0.63) in the healthy individuals and patients with EH, respectively.

The analysis of concentrations of cytokines and vasoactive peptides measured at different times of day yielded strong direct correlations between the evening concentrations of IL1α and ADMA, $r = 0.76$, $p < 0.01$ (for their diurnal levels $r = 0.58$, $p < 0.05$); sLIFr and SDMA, $r = 0.85$, $p < 0.001$ (for their diurnal levels $r = 0.69$, $p < 0.05$); beM-CSF and SDMA, $r = 0.81$, $p < 0.01$ (for their diurnal levels $r = 0.51$, $p < 0.05$). The strength and the direction of correlations between the levels of IL1β, IL1ra, IL18, IL18BP, IL37, IL6, sIL6r, LIF, TNFα, sTNF-RI, IL2, IL10, TGF-β1, IL8, CX3CL1, CXCL10, INFγ, and the concentrations of vasoactive peptides in the blood serum measured in the

evening did not differ from the results obtained for the samples that were collected at 7:00 a.m.

A pronounced increase in the concentrations of the cytokines that correlated with vasopressor factors in patients with stage II EH suggests that there might be a correlation between the morning/evening levels of these cytokines and the BP phenotypes (dipper, non-dipper and night-peaker).

The dippers, whose BP dropped by normal 10–20% at nighttime, as well as other patients with stage II EH, had higher concentrations of proinflammatory cytokines. In this subgroup, the dynamics observed for IL1β, IL18, IL18BP, IL37, LIF, sLIFr, TNFα, sTNF-RI, IL2, IL8, IL10, IFNγ, CX3CL1, CXCL10, TGFβ1, and M-CSF were the same as in the healthy individuals (Table 2). Only 6 cytokines demonstrated a different behavior: IL6 and sIL6r (a less pronounced rise in IL6 in the evening accompanied by high and stable sIL6r concentrations); protective IL1ra and erythropoietin that did not decline as in the healthy volunteers; elevated evening IL1α that demonstrated a more than 20% rise in comparison with its morning concentrations. The analysis of the cyclic dynamics demonstrated by 21 studied cytokines established that evening deviations from morning concentrations by more than 20% were the most pathogenic in terms of their contribution to the progression of hypertension. In the dipper group, such deviations were observed only for IL1α associated with increased ADMA in patients with stage II EH.

The non-dippers, whose BP dropped by only 10% at the most, demonstrated a more pronounced change in cytokine concentrations in the evening than the healthy controls and the dippers (Table 2). Only 9 cytokines followed a similar pattern

Table 1. Morning and evening concentrations of cytokines (pg/ml) in the serum samples of patients with stage II EH (duration of 10 to 14 years, no antihypertensive treatment applied)

Cytokines	Healthy controls ($n = 50$)		Patients with stage II EH ($n = 90$)	
	7.00 a.m.	8:00 p.m.	7.00 a.m.	8:00 p.m.
	1	2	3	4
IL1β	5.2 (1.87)	5.95 (1.37)['1]	18.7 (5.8)[*1]	22.8 (6.12)[*2,3]
IL1α	3.83 (1.17)	4.37 (0.98)['1]	13.2 (3)[*1]	17.2 (2.93)[*2,3]
IL1ra	691 (99)	598 (96)[^1]	575 (108)[*1]	582 (96.2)
IL18	197 (59)	201 (48)	371 (84)[*1]	382 (83)[*2]
IL18BP	4934 (1173)	4903 (1122)	6710(1980)[*1]	5970 (1783)[^2,3]
IL37	90 (25.6)	88 (17.3)	92 (24.5)	71.6 (18.2)['1]
IL6	3.12 (0.63)	4.03 (0.69)[*1]	23.7 (4.2)[*1]	27.2 (3.92)[*2,3]
sIL6r	681 (52)	478 (50)[*1]	1826 (263)[*1]	1902 (255)[*2]
LIF	1.41 (0.61)	1.47 (0.54)	7.54 (2.4)[*1]	9.08 (2.24)[*2,3]
sLIFr	3920 (1123)	4003 (1223)	4100 (1200)	5100 (1420)[*2,3]
TNF-α	7.56 (1.83)	8.49 (1.64)[^1]	21.4 (4)[*1]	23.2 (3.84)[*2^3]
sTNF-RI	1620 (367)	1817 (367)['1]	2770 (670)[*1]	2810 (627)[*2]
IL2	10.1 (2.12)	10.7 (1.97)	10.8 (3.11)	10.4 (2.82)
IL8	8.75 (1.82)	9.21 (2.03)	29 (7.83)[*1]	31.2 (6.61)[*2]
IL10	18.2 (5.7)	15.9 (4.83)[^1]	26.2 (7.9)[*1]	22.3 (6.87)[*2'3]
IFNγ	8.97 (2.23)	8.84 (2.14)	18.4 (4.3)[*1]	18.7 (3.96)[*2]
M-CSF	202 (51)	197 (47)	389 (92)[*1]	463 (87)[*2,3]
CX3CL1	422 (61.3)	436 (58.1)	520 (120)[*1]	503 (112)[^2]
CXCL10	8.83 (1.73)	8.97 (1.24)	18.2 (4.5)[*1]	17.9 (3.84)[*2]
TGFβ1	11.5 (3.23)	10.2 (2.84)	21.4 (4.64)[*1]	20.2 (4.24)[*2]
Erythropoietin	3.93 (1.31)	4.27 (1.01)	12.6 (3.8)[*1]	17.2 (3.43)[*2,3]

Note: significance of differences: ' — < 0.05, ^ — < 0.01, * — < 0.001 (comparison with the specified group). The data are presented as an arithmetic mean (M) and a standard deviation (SD).

of morning-to-evening changes as in the healthy controls, including IL18, TNFα, sTNF-RI, IL2, IL8, IFNγ, TGFβ1, CX3CL1, and CXCL10. Evening deviations from morning concentrations reaching over 20% were observed for IL1β, IL1α, IL37, LIF, sLIFr, IL10, M-CSF, and erythropoietin and were statistically significant between the non-dippers and the healthy controls. IL37 and IL10, which might be protective against the progression of EH, as suggested by the study results, demonstrated a more pronounced evening drop in the patients with insufficiently low nocturnal BP than in the healthy individuals. In contrast, IL1β, IL1α, LIF, sLIFr, M-CSF, and erythropoietin concentrations tended to grow in the evening in such patients. It should be noted that evening and morning levels of IL1ra in the non-dippers did not differ, whereas a significant IL1ra decline was observed in the controls, suggesting protection against rising IL1β and IL1α, just like in the dippers.

In the night-peakers with elevated nocturnal BP, the daily dynamics of IL1ra, IL18, TNFα, sTNF-RI, IL2, IL8, CX3CL1, CXCL10, and TGFβ1 were similar to those observed in the controls (Table 2). Evening deviations from morning concentrations reaching over 20% were noticed for IL1β, IL1α, IL37, LIF, sLIFr, M-CSF, neopterin, and erythropoietin and were statistically significant between the night-peakers and the healthy controls. IL37, which could be relatively protective against the progression of hypertension in patients with elevated BP, exhibited a more pronounced evening decline as compared to the controls, whereas for IL1β, IL1α, LIF, M-CSF, neopterin and erythropoietin, a more pronounced rise

was observed. It should be noted that in the night-peakers, IL10 concentrations did not differ between morning and evening, although they did go down in the healthy controls; this tendency might be protective against the rising levels of proinflammatory cytokines. In our study, the cytokine synthesis pattern was similar in the night-peakers and the non-dippers, differing only in the behavior of IL1ra. The evening concentrations of this cytokine dropped significantly in the night-peakers; however, proinflammatory IL10 did not drop at all, which was not the case with the non-dippers. This makes the night-peaker and non-dipper groups somewhat equivalent in terms of protection against EH progression. In the night-peakers, the evening levels of IL1α, M-CSF and erythropoietin increased more than in the non-dippers (> 35% at 8:00 p.m.), but the overall pattern of their dynamics was the same.

DISCUSSION

Cytokines, including IL1β, TNFα, IFNγ, IL6, IL4, and IL10, affect the biological activity of neurotransmitters/neuromodulators and exert a direct effect on neurons in some areas of the brain, regulating the sleep cycle, which remains the most well-studied circadian rhythm [2]. This means that these cytokines are involved in the processes indirectly related to the sleep/wake cycle, including blood pressure fluctuations. We have found that the daily dynamics of cytokine concentrations is abnormal in patients with stage II EH (duration of 10 to 14 years) in comparison with healthy controls: concentrations of somnogenic

Table 2. The relationships between morning and evening concentrations of cytokines (pg/ml) in the serum samples of patients with stage II EH (duration of 10 to 14 years, no antihypertensive treatment applied) AND the blood pressure phenotype

Cytokines	«Dipper» (n = 36)		«Non-dipper» (n = 30)		«Night-peaker» (n = 24)	
	7.00 a.m.	8:00 p.m	7.00 a.m.	8:00 p.m	7.00 a.m.	8:00 p.m
	1	2	3	4	5	6
IL1β	16.3 [3.24–25.1]	18.7 [10.3–29.4]^1	16.8 [3.32–26.4]	22.3 [15.6–32.8]^2*3	22.4 [10.4–32.2]*1,3	29.4 [18.9–36.3]*2,5^4
IL1α	12.5 [5.72–17.3]	15.2 [10.4–21.3]*1	12.8 [5.23–17.9]	17.3 [13.8–23.1]^2*3	16.2 [10.1–20.2]*1,3	23.7 [17.2–24.8]*2,5,4
IL1ra	570 [493–670]	591 [517–691]	567 [488–691]	586 [504–652]	589 [468–702]	514 [387–601]'2,4,5
IL18	370 [311–432]	400 [322–465]	362 [291–421]	407 [319–467]	389 [298–444]	397 [263–450]
IL18BP	6740 [5370–8457]	5990[4700–7100]^1	6940 [5128–8620]	6103[4620–7130]'3	6630 [4970–8520]	5930 [4550–7310]'5
IL37	87.5 [70.2–110]	82.3 [59.3–109]	90.1 [67.5–106]	60.4 [32.2–83.3]*2,3	85.4 [68.7–114]	62.5 [33.4–80.9]*2,5
IL6	23.9 [20.8–26.7]	27.2 [24.5–29.9]^1	24.3 [20–26.9]	27.7 [24 –29.3]^3	23.7 [21.2–26.5]	26.8 [24.8–30]^5
sIL6r	1826 [1648–2003]	1902 [1730–2073]	1793 [1615–2121]	1837 [1710–2113]	1804 [1583–2107]	1924 [1698–2107]
LIF	8.07 [4.68–10.3]	8.56 [6.86–9.93]	7.09 [4.92–9.12]	10.8 [9.92–12.1]^2*3	8.32 [5.13–9.3]	11.3 [8.43–12.9]*2,5
sLIFr	4140 [3120–4820]	4320 [3270–5240]	4060 [3230–4770]	5930 [4600–7720]*2,3	3980 [3040–4910]	6030 [4580–7810]*2,5
TNF-α	21.2 [18.3–24]	23.6 [20.9–26.1]^1	20.7 [18.9–23.5]	23.1 [20.2–25.7]^3	20.9 [17.9–24.3]	23.9 [21.6–26.7]^5
sTNF-RI	2790 [2320–3220]	3030 [2607–3450]'1	2810 [2280–3110]	3110 [2406–3640]'3	2720 [2120–3310]	3090 [2540–3620]'5
IL2	10.6 [8.92–12.9]	10.4 [8.5–12.2]	10.9 [8.83–12.4]	11.1 [8.69–12.5]	10 [8.63–11.7]	10.4 [8.57–12.1]
IL8	29.1 [23.7–34.3]	31.2 [26.8–35.6]	30.4 [23.9–34.8]	32.4 [27.7–36.2]	29.8 [22.9–33.8]	30.9 [25.9–35.3]
IL10	26.2 [20.9–31.5]	22.3 [17.7–27]'1	27.8 [19.7–32.6]	21.6 [16.9–26.3]'3	25.7 [19.1–30.3]	23.5 [18.5–28.1]
IFNγ	18.4 [15.5–21.3]	19.3 [16–21.8]	18.9 [16.7–22.3]	20.2 [17.3–22.4]	17.9 [14.7–21.8]	21.1 [17.4–24.3]'2,5
M-CSF	371 [320–448]	402 [345–484]'1	378 [314–452]	451 [391–540]^2*3	394 [322–440]	517 [410–614]*2,4,5
CX3CL1	520 [439–607]	503 [427–579]	553 [455–647]	544 [409–597]	546 [461–639]	519 [447–602]
CXCL10	18.6 [15.2–21.3]	17.7 [15.1–20.5]	19.5 [16.3–22.1]	18.2 [15.8–21.3]	17.9 [14.7–21.9]	18.9 [15.4–21.3]
TGFβ1	21.4 [18.3–24.9]	20.3 [17.2–23.1]	22.1 [18.1–24.3]	19.7 [16.8–22.9]	20.9 [17.9–24.5]	20.6 [17.7–23.3]
Erythropoietin	15.5 [10.2–20.3]	15.7 [9.62–19.9]	12.5 [9.12–15.6]^1	17.2 [12.9–22.3]^2*3	12.7 [7.8–16.9]^1	20.3[16.2–24.7]*2,5^4

Note: significance of differences: ' — < 0.05, ^ — < 0.01, * — < 0.001 (comparison with the specified group); 1 — 7:00 a.m., dippers, 2 — 8:00 p.m, dippers, 3 — 7:00 a.m., non-dippers, 4 — 8:00 p.m., non-dippers, 5 — 7:00 a.m., night-peakers, 6 — 8:00 p.m, night-peakers. The data are presented as a median (Me) and an interquartile range [C25-C75].

IL1β and IL1α and a few other cytokines whose circadian dynamics remain understudied (LIF, sLIFr and M-CSF) significantly increase in the evening in hypertensive patients. In healthy individuals, evening concentrations of LIF, sLIFr, and M-CSF undergo a less pronounced rise (<10%), whereas IL1α and erythropoietin increase by 10–15%. Importantly, patients with stage II EH (duration of 10-14 years, no antihypertensive therapy) demonstrate a more than 20% increase in the evening levels of IL1α, LIF, sLIFr, M-CSF, and erythropoietin. Such high values correlate with a rise in the concentrations of vasopressor peptides, in particular with ADMA and SDMA. ADMA competes with L-arginine for binding to eNOS [11]. SDMA is a structural ADMA isomer that has a role in arginine binding to a transmembrane transporter by limiting its availability to eNOS and curbing the subsequent synthesis of NO [12]. A substantial increase in the evening levels of the mentioned cytokines contributes to EH progression, which has been confirmed in a number of recent experimental studies. IL1α has a direct stimulating effect on the proliferation of vascular smooth muscle cells, activates fibrosis and enhances the expression of angiotensin II and endothelin I receptor mRNA on the surface of the smooth muscle cells of the vascular wall [13]. Previously, we reported that IL1α caused a secondary increase in LIF levels in the blood of patients with EH [14], which can stimulate the LIF-mediated influx of Ca^{2+} and ERβ-dependent activation of MAPK [15]. Growing concentrations of M-CSF inhibit eNOS activity [16] and suppress the mRNA expression of its own receptor IL34 (PTP-ζ) [17]. These findings are consistent with our experimental data. A decline in IL34 concentrations occurring in the background of rising M-CSF [18] inhibits its protective effect on the brain. Together, these processes can cause vasodilation and increase the sensitivity of blood vessels to vasopressors. They unfold in the background of declining IL37 concentrations (the cytokine has a protective effect in patients with EH) and stable IL10 levels and are more pronounced in individuals with a pathological daily BP cycle (non-dippers and night-peakers).

In the dippers whose BP dropped at nighttime by over 10%, the studied cytokines, including IL1β, IL18, IL18BP, IL37, LIF, sLIFr, TNFα, sTNF-RI, IL2, IL8, IL10, IFNγ, CX3CL1, CXCL10, TGFβ1, and M-CSF, followed the same pattern as in healthy individuals: except for IL6/sIL6r and erythropoietin, all these cytokines underwent a less than 15% drop in the evening. A more than 20% increase in IL1β, IL1α, LIF, sLIFr, M-CSF and erythropoietin concentrations registered in the evening is a potential isofactor that changes the tone of blood vessels and interferes with blood pressure regulation by the central nervous system. Under these circumstances, blood pressure does not decline sufficiently at night, and evening concentration of the mentioned cytokines rise by 35%, which is associated with elevated nocturnal BP. These changes can be explained by the established correlations with vasopressor factors and are consistent with the experimental data provided above. Changes in the protective cytokine mechanisms that distinguish healthy individuals from patients with EH illustrate resistance to the effect of proinflammatory cytokines. In dippers, as well as in healthy volunteers, IL10 concentrations decline in the evening, but IL1ra does not go down, which is a compensatory antagonistic effect against growing IL1β and IL1α (sIL6r does not decline in antihypertensive patients but does decrease in healthy individuals), which adds to the activity of IL6. In non-dippers, evening concentrations of IL37 display a negative dynamic. In night-peakers, IL10 does not decline in the evening, suggesting a compensatory effect against the rising proinflammatory cytokines; however, IL1ra levels do drop in this group of patients.

CONCLUSIONS

We have identified a number of changes in the daily dynamics of peripheral blood cytokine concentrations in patients with stage II EH that are associated with abnormal daily BP cycles (non-dippers and night-peakers). Here, the major contribution is done by a more than 20% rise in LIF, sLIFr, M-CSF, IL1α, and erythropoietin. In the night-peaker group, the rise is over 35%. Patients with a sufficient drop in BP at night (dippers) have higher concentrations of proinflammatory cytokines than healthy controls but the overall daily dynamics are the same in these two groups.

Understanding the pathophysiological role of cytokine levels and their fluctuations over a 24-h cycle in patients with stage II EH could inspire new methods for EH prevention, reducing end-organ damage and tailoring treatment strategies to an individual patient. This proves the importance of research into the dynamics of physiological parameters with respect to circadian rhythms.

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