Circadian Dynamics of Monocyte Phagocytic Activity in Women during Lactation Complicated by Iron Deficiency

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 7, pp. 35-38, July, 2005
Original article submitted November 22, 2004

Biorhythms of phagocytic activity of peripheral blood monocytes were studied during lactation in healthy women and in women with iron deficiency. Circadian organization of monocyte function was characteristic of healthy nursing women. Strain in the system was associated with elevation of the mean circadian values for reserve potential of the absorption and digestive capacity of phagocytes. Circadian rhythms of the phagocytic and digestive capacity of peripheral blood monocytes were leveled during lactation complicated by iron deficiency. Decreased coefficient of the parameters activation is an early manifestation of latent iron deficiency. Decrease in the basic function parameters and reserve potential are directly related to the decrease in serum ferritin concentration.

Key Words: *biorhythms; lactation; monocytes; phagocytosis; iron deficiency*

During recent years the incidence of iron deficiency anemia (IDA) in pregnant women and children in Russia increased 3- and 2.2-fold, respectively [1]. The prevalence of iron deficiency in risk groups (pregnant and breast-feeding women, infants and adolescents) reaches 80%. Deficiency of iron-containing enzymes (a manifestation of sideropenia) is responsible for functional insufficiency of phago- and immunocytes [9]. Macrophages (obligate components of breast milk) participating in the regulation of iron metabolism and integration of nonspecific and specific defense mechanisms [2,3] regulate immunity in babies [8]. High sensitivity to endo- and exogenous factors of spatial and temporal organization of functional systems [4,5] and high informative value of methods for chronodiagnosis of latent forms of iron deficiency [2] prompted us to evaluate the circadian dynamics of phagocytic activity of monocytes during lactation and determine the rhythmometric criteria of their dysfunction in breast-feeding women.

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MATERIALS AND METHODS

A total of 200 women were examined during month 6 of lactation: 136 healthy breast-feeding women, 36 with latent iron deficiency (LID), and 28 with IDA (20-32 years). Control group consisted of 35 healthy nonpregnant non-breast-feeding women of reproductive age (HWRF; 18-30 years).

Blood levels of erythrocytes and hemoglobin were evaluated on a Cell-Dyn 1700 hemanalyzer. Serum concentration of ferritin was measured by enzyme immunoassay and iron by diphenylphenanthroline method (Lachema). Diurnal excretion of the trace element was estimated by its urinary concentration with consideration for the 24-h diuresis. MDA concentration was evaluated as described previously [6], α-tocopherol level by a previously described method [7]. The phagocytic capacity of peripheral blood monocytes was evaluated by phagocytosis of latex particles: by the percentage of phagocytizing cells (phagocytic index) and mean number of phagocytosed particles per active cell (phagocytic number). Digestive capacity of the cells was evaluated in the NBT

0007-4888/05/1401-0029

test by the percentage of NBT-positive monocytes and mean number of formazane granules per active cell (digestive activity). Stimulated tests of the phagocytizing and digestive capacity of monocytes were reproduced using pyrogenal (25 μg/ml). Activation coefficients were calculated as the ratio of stimulated to spontaneous test values. Blood for analyses was collected from the ulnar vein, the parameters were

TABLE 1. Circadian Dynamics of Serum Ferritin Concentrations and Parameters of Phagocytic Capacity of Peripheral Blood Monocytes during Lactation (*M*±*m*)

Time	SF, μg/liter	PI, %	PI AC	PN	PN AC
Healthy women of reproductive age					
6:00	38.8±1.9	38.60±1.42*	3.05±0.09*	1.77±0.06*	3.52±0.23*
12:00	42.2±2.2*	40.80±0.93	2.38±0.13	4.75±0.16	2.45±0.15
18:00	39.6±2.2	59.60±1.12*	1.28±0.12*	7.56±0.19*	1.07±0.11*
24:00	27.0±1.8*	45.80±0.74	1.69±0.09	4.60±0.12	1.32±0.09
Mesor	36.9±3.3	46.20±1.01	2.10±0.11	4.67±0.17	2.09±0.17
Amplitude	7.5±2.7	10.50±0.74	0.89±0.06	1.45±0.19	1.22±0.14
% of mesor	20.3	22.7	42.3	31.5	58.3
Acrophase, h	12:16	18:43	7:09	18:13	6:39
	11:18; 14:51	17:18; 19:23	6:14; 8:25	17:54; 19:45	5:14; 8:10
Healthy breast-feeding women					
6:00	30.6±2.2	38.70±1.42*	3.45±0.12*	5.87±0.18*	4.78±0.15*
12:00	47.2±3.1*	42.90±0.73	3.05±0.08	7.30±0.19	2.89±0.16
18:00	37.5±2.9	63.70±1.12*	2.59±0.09*	8.10±0.18*	1.32±0.09*
24:00	26.0±3.3*	47.90±0.74	2.88±0.11	7.45±0.18	2.25±0.04
Mesor	34.7±3.7	48.3±1.3	2.99±0.16^{+}	7.18±0.20^{+}	2.81±0.19^{+}
Amplitude	20.6±3.1	12.50±1.08	0.43±0.12	1.12±0.16	1.73±0.18
% of mesor	59.4	25.8	14.4	15.5	61.5
Acrophase, h	11:33	18:07	6:15	17:56	5:56
	10:18; 13:31	16:49; 19:31	5:55; 8:28	17:11; 19:05	5:8; 7:45
Breast-feeding women with LID					
6:00	9.8±2.5	36.78±1.25	1.49±0.13	3.89±0.82	1.35±0.16
12:00	11.3±3.4	40.78±1.58	1.95±0.15	4.56±0.59	1.75±0.25
18:00	12.6±3.8	45.45±1.24	2.15±0.11	5.23±0.25	1.79±0.28
24:00	10.7±3.7	43.96±1.36	1.58±0.12	1.89±0.39	1.02±0.13
Mesor	11.1±4.7^{o}	40.99±1.13^{o}	1.79±0.12^{o}	3.89±0.92^{o}	1.48±0.28^{o}
Amplitude	4.6±1.0	4.34±1.56	0.33±0.10	1.67±0.86	0.39±0.27
% of mesor	18.1	10.5	18.4	42.9	26.3
Acrophase, h	No rhythm	No rhythm	No rhythm	No rhythm	No rhythm
Breast-feeding women with IDA					
6:00	8.4±2.8	40.88±1.25	1.10±0.13	3.78±0.80	1.26±0.16
12:00	9.6±3.2	42.88±1.58	1.53±0.15	4.45±0.45	1.36±0.25
18:00	10.2±5.5	45.55±1.24	1.70±0.11	5.12±0.32	1.70±0.28
24:00	9.0±2.6	44.06±1.36	1.16±0.12	1.78±0.23	1.23±0.13
Mesor	9.3±5.3^{o}	43.09±1.13^{o}	1.37±0.12^{o}	3.78±0.92^{o}	1.39±0.28^{o}
Amplitude	3.8±1.1	2.34±1.56	0.3±0.1	1.67±0.86	0.23±0.07
% of mesor	11.8	5.4	21.9	44.2	16.5
Acrophase, h	No rhythm	No rhythm	No rhythm	No rhythm	No rhythm

Note. CF: serum ferritin; PI: phagocytic index; PN: phagocytic number; AC: activation coefficient. Here and in Table 2: $p<0.05$ compared to *mesor, $^{+}$parameters in healthy women of reproductive age, ^{o}healthy breast-feeding women.

measured 4 times during 24 h (at 6.00, 12.00, 18.00, and 24.00).

Decrease in serum ferritin concentration <30 μg/liter and iron <12 μmol/liter in the presence of normal erythrocyte and hemoglobin levels served as criteria of LID. Decreased reserve, transport, and hemoglobin funds of the trace element indicated IDA.

The results were processed using Statgraphics and Kosinor software. The significance of differences was evaluated using Student's *t* test.

TABLE 2. Circadian Dynamics of NBT-Positive Peripheral Blood Monocytes during Lactation (*M*±*m*)

Time	NBT-positive monocytes, %	AC, % of NBT-positive monocytes	Monocyte DA	DA AC
Healthy women of reproductive age				
6:00	38.19±0.81*	2.58±0.03*	1.56±0.09*	2.32±0.03*
12:00	43.56±0.75	1.32±0.06	3.07±0.18	1.36±0.06
18:00	56.74±0.45*	1.28±0.02*	4.62±0.15*	1.15±0.09*
24:00	40.87±0.31	1.86±0.08	3.83±0.18	1.65±0.08
Mesor	44.85±0.87	1.76±0.07	3.27±0.23	1.69±0.07
Amplitude	9.28±0.63	0.65±0.04	1.53±0.19	0.59±0.04
% of mesor	20.7	36.9	46.7	34.9
Acrophase, h	19:54	06:27	20:15	06:11
	18:10; 20:15	05:32; 07:21	17:32; 21:05	05:12; 07:28
Healthy breast-feeding women				
6:00	48.68±1.15	3.28±0.07*	3.61±0.19	3.06±0.03*
12:00	41.01±0.74*	2.09±0.12	2.00±0.21*	1.02±0.08*
18:00	61.86±1.45*	1.82±0.11*	4.86±0.13*	1.25±0.11*
24:00	48.86±1.75	2.36±0.13	3.75±0.16	1.69±0.04
Mesor	49.94±1.12	2.39±0.10^{+}	3.56±0.16^{+}	2.01±0.09^{+}
Amplitude	10.42±0.74	0.73±0.09	1.43±0.18	1.02±0.09
% of mesor	20.8	30.5	40.1	50.7
Acrophase, h	19:41	6:35	19:18	6:27
	18:22; 20:35	5:52; 7:48	18:52; 20:47	5:22; 7:36
Breast-feeding women with LID				
6:00	44.68±1.48	1.38±0.11	1.48±0.28	1.03±0.12
12:00	46.98±1.45	1.39±0.08	1.80±0.18	0.92±0.13
18:00	45.12±2.12	1.18±0.02	1.93±0.15	0.95±0.19
24:00	47.01±1.36	1.16±0.05	2.12±0.19	1.32±0.66
Mesor	45.99±1.61	1.28±0.06^{o}	1.81±0.22^{o}	1.06±0.09^{o}
Amplitude	1.17±0.56	0.12±0.05	0.32±0.25	0.20±0.06
% of mesor	2.5	9.3	17.6	18.8
Acrophase, h	No rhythm	No rhythm	No rhythm	No rhythm
Breast-feeding women with IDA				
6:00	42.18±1.48	1.07±0.11	1.28±0.28	0.93±0.12
12:00	44.48±1.45	1.08±0.08	1.60±0.18	0.82±0.13
18:00	43.62±2.12	0.88±0.02	1.73±0.15	0.85±0.19
24:00	43.61±1.36	0.86±0.05	1.62±0.19	1.01±0.16
Mesor	40.49±1.61^{o}	0.98±0.12^{o}	1.21±0.22^{o}	0.91±0.19^{o}
Amplitude	1.15±0.56	0.10±0.01	0.22±0.05	0.11±0.06
% of mesor	2.8	10.2	18.1	12.1
Acrophase, h	No rhythm	No rhythm	No rhythm	No rhythm

Note. DA: digestive activity.

RESULTS

Increased functional activity of monocytes is typical of the lactation period. This is seen from a significant increase in the reserve potential for phagocytic and digestive capacities and decreased amplitudes of phagocytic index and NBT-positive monocyte activation coefficients (Tables 1, 2). Boundaries of confidence intervals for acrophases of the monocyte phagocytic and digestive capacity coincided during rather long intervals, the active phase for these parameters being prolonged during the evening hours. Circadian fluctuations in the characteristics of reserve potential and spontaneous activity of monocytes were in the counterphase. Reciprocal time ratios of these parameters indicate high bactericidal potential of phagocytes, as according to the "initial values law" [10], any function can be modified in the opposite direction mostly during the period of its maximum (positive or negative) deviation from the mean values.

Analysis of the temporal characteristics of iron metabolism and monocyte function revealed significant relationship between these parameters: the peak of phagocytic activity was regularly followed by increase in digestive capacity (Tables 1, 2). Exocytosis of Fe^{2+} by macrophages and LPO potentiated by them caused an increase in the plasma level of free iron (acrophase 00.15: 23.17, 02.21) and MDA during the night hours (acrophase 04.15: 22.15, 05.25), which determined the peak of urinary excretion of iron and MDA.

Circadian rhythms of phagocytic and digestive capacities of the cells were leveled, the parameters of their reserve potential were reduced (Tables 1, 2). A significant correlation between the levels of serum ferritin and parameters of monocyte functional activity was detected: r=+0.81 (p<0.05) for phagocytic index, r=+0.73 (p<0.05) for phagocytic number, r=+0.62 (p<0.05) for the percentage of NBT-positive monocytes, and r=+0.97 (p<0.05) for digestive activity. This correlation was the most pronounced for the reserve potential parameters. Hence, phagocytic activity of monocytes can serve as the criterion of tissue iron fund status. The earliest signs of iron deficiency are leveling of circadian rhythms for the parameters of cell phagocytic and digestive capacity and decrease of the mean circadian coefficients of their activation.

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