Erythropoiesis and Iron Metabolism Biorhythms in Children with Chronic Pyelonephritis

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Circadian dynamics of ferritin, serum iron, and MDA concentrations, ineffective, normal, and terminal kinetic populations of the erythron were studied in healthy girls aged 7-9 years and girls suffering from chronic pyelonephritis. The production of highly active macrocyte population, descending from terminal erythropoiesis, was increased during pyelonephritis remission, which determined reduction of serum iron concentration during the morning hours and leveling of its circadian rhythm because of high utilization of the trace element. Progressive reduction of erythrocyte count and hemoglobin content during the active phase of pyelonephritis correlated with the increase in the population of microcytes with low activity of glucose-6-phosphate dehydrogenase and short life span, paralleled by an appreciable increase in ferritin and MDA concentrations during the evening hours. Stimulation of alternative erythron kinetic types (terminal and ineffective) underlies these changes.

Key Words: *biorhythms; erythropoiesis; iron metabolism; lipid peroxidation; pyelonephritis*

Anemia is one of the main syndromes in patients with chronic renal diseases [4-6]. A shorter half-life period of erythrocytes and their greater sensitivity to oxidative stress are observed even in cases with retained glomerular filtration [2,7]. Disorders in iron utilization and inhibited synthesis of heme in erythrocyte precursor mitochondria are hypothesized to promote the formation of short-lived erythrocytes [5,7]. The absence of data on the specific features of the erythron kinetics and its contribution to the pathogenesis of anemia in children with chronic pyelonephritis (CPN) and the informative value of biorhythmological criteria for the early diagnosis of iron metabolism disorders [1,3] prompted us to investigate the regularities of disorders in erythropoiesis and iron metabolism biorhythms and to develop, on the base of our results, criteria for prediction and early diagnosis of anemias in children with CPN.

MATERIALS AND METHODS

A total of 86 girls aged 7-9 years were examined; 42 of them belonged to health group 1 according to comprehensive evaluation of the health status and 44 suffered from CPN (29 with remission and 15 with exacerbation stage).

Common clinical nephrological studies were carried out in all children. The content of hemoglobin, erythrocytes, and their volume distribution were evaluated on a Cell-Dyn 3500 automated analyzer (Abbott). Reticulocyte content was evaluated after staining of smears with 1% brilliant cresyl blue. Activity of glucose-6-phosphate dehydrogenase (G-6-PDH) was measured by quantitative cytochemistry for estimation of populations of erythrocytes with low (0-3 granules of reduced formasane), medium (7-9 granules), and high (10-14

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granules) activities of the enzyme, intrinsic of descendants from ineffective, normal, and terminal types of the erythron kinetics, respectively. Normal values of these parameters are 8-15, 75-78, and 5-10%, respectively [1,5]. In parallel, daily production and life span of erythrocytes and MDA content were evaluated. Serum iron concentration was measured by the bathophenanthroline method (LACHEMA), ferritin concentration was determined by radioimmunoassay (IRNO-ferritin). Transferrin saturation coefficient (TSC) was calculated. Blood for analysis was collected from the ulnar vein, the parameters were analyzed daily at 6.00, 12.00, 18.00, and 24.00.

The significance of differences was evaluated using Student's *t* test.

RESULTS

Circadian periodicity with midday peak is characteristic of fluctuations of hemoglobin concentration and erythrocyte content in healthy girls (Table 1). The highest levels of erythrocytes and reticulocytes in this group were recorded during the evening and night hours. The predominant volume of normocytes was recorded for the medium active (by G-6-PDH level) population (Table 1). The maximum of highly active macrocyte population was regularly observed at noon, while low-active microcytes exhibited their maximum activity in the evening, when the highest concentrations of serum ferritin were recorded.

Synchronization of the dynamics of reticulocytes, normocytes, and medium active (by G-6-PDH) erythrocyte population persuasively demonstrates that the night hours are characterized by normal kinetics, while reduced level of sideremia and TSC indicate the highest utilization of iron for hemoglobin biosynthesis in the main population (normocytes), characterized by the longest life span (Table 1).

The maximum concentration of serum ferritin is typical of the evening and night hours (Table 2).

TABLE 1. Rhythmometric Parameters of Erythropoiesis in Girls with CPN (*M*±*m*)

Group	Parameter	Mesor	Amplitude	Acrophase (95% confidence interval)
Healthy girls	Erythrocytes, $\times10^{12}$/liter	4.1±0.1	0.8±0.1	10.19 (09.15; 12.31)
(health group 1)	Hemoglobin, g/liter	134.2±1.8	9.1±0.2	09.40 (09.00; 12.11)
	Reticulocytes, $\times10^{9}$/liter	42.2±2.1	12.3±2.1	01.45 (23.12; 03.41)
	Erythrocyte production, $\times10^{9}$/liter	67.5±5.4	39.1±2.1	00.53 (23.00; 04.17)
	Erythrocyte life span, days	75.5±2.1	27.1±1.9	01.50 (23.50; 03.45)
	Low-active microcytes, %	12.5±0.5	6.3±0.9	21.12 (20.30; 21.50)
	Medium-active normocytes, %	74.6±0.7	21.1±1.1	01.31 (24.01; 04.11)
	Highly active macrocytes, %	12.9±0.3	7.3±0.7	12.03 (09.11; 12.31)
CPN remission	Erythrocytes, $\times10^{12}$/liter	3.9±0.1	0.6±0.1	9.50 (08.50; 12.50)
	Hemoglobin, g/liter	132.1±2.0	8.7±0.2	9.10 (08.30; 11.52)
	Reticulocytes, $\times10^{9}$/liter	28.7±3.1*	8.7±0.6	02.17 (00.30; 04.57)
	Erythrocyte production, $\times10^{9}$/liter	77.3±6.1	15.3±4.1	No rhythm
	Erythrocyte life span, days	55.6±3.7*	19.3±2.1	02.21 (00.41; 02.11)
	Low-active microcytes, %	19.3±0.7*	5.4±1.1	19.10 (18.30; 21.00)
	Medium-active normocytes, %	54.9±1.1*	15.3±1.8	00.40 (23.30; 02.51)
	Highly active macrocytes, %	25.8±0.5*	10.3±2.1	11.30 (08.30; 12.50)
CPN exacerbation	Erythrocytes, $\times10^{12}$/liter	3.5±0.3*	0.4±0.3	No rhythm
	Hemoglobin, g/liter	110.7±0.4	5.3±0.4	No rhythm
	Reticulocytes, $\times10^{9}$/liter	21.1±2.9*	8.5±0.4	01.41 (00.20; 03.41)
	Erythrocyte production, $\times10^{9}$/liter	56.9±5.7*	12.7±3.4	No rhythm
	Erythrocyte life span, days	47.9±3.1*	18.7±3.1	02.41 (00.17; 03.11)
	Low-active microcytes, %	31.8±3.6*	6.3±2.1	No rhythm
	Medium-active normocytes, %	35.1±2.9*	9.8±3.0	No rhythm
	Highly active macrocytes, %	32.1±3.1*	8.6±3.2	No rhythm

Note. Here and in Table 2: *$p<0.05$ compared to healthy girls.

Ferritin and MDA concentrations were in strict direct correlation throughout the 24 hours (r=0.92; p<0.05), this indicating a close functional relationship between these parameters.

Hence, analysis of the spatial and time organization of erythropoiesis and iron metabolism in healthy girls demonstrated a relationship between circadian rhythms of sideremia, on the one hand, and erythron kinetics and LPO status, on the other, this suggesting the use of these parameters as informative criteria for the diagnosis of iron metabolism disorders.

The rhythmometric values of the content of erythrocytes and hemoglobin concentrations in girls with CPN remission were the same as the values intrinsic of healthy girls (Table 1). Elevation of MDA concentration initiated deep restructuring of the erythron kinetics at the expense of significant increase in macrocyte population with high G-6-PDH activity, increment in microcytes with low activity of the enzyme, and decrease in the normocyte fraction (Table 2). Stimulation of terminal erythropoiesis under conditions of oxidative stress should be regarded as an indicator of adaptive strain of physiological glutathione system [9]. The fraction of highly active macrocytes is characterized by the greatest potential of antiradical and antioxidant defense and the shortest life span [1,6].

Hence, leveling of the circadian rhythm of 24-h production of erythrocytes is caused by restructuring of the erythron kinetics. Reduction of serum iron concentration and TSC at noon is caused by increased production of macrocytes with high G-6-PDH activity, which leads to leveling of the circadian rhythm of sideremia, indicating functional deficiency of iron. The increase of ferritin concentration associated with it is obviously caused by more intense phagocytosis of short-lived erythrocytes and blockade of iron release from active macrophages [10].

Increase in ferritin concentration and a decrease in TSC, mean 24-h hemoglobin values, and erythrocyte production during CPN exacerbation were paralleled by elevation of MDA concentration and increase in the population of short-lived microcytes, as well as by leveling of the circadian rhythm of these parameters (Tables 1, 2). Reduced deformability of short-lived microcytes promoted stimulation of erythrophagocytosis and increased macrophage secretion of IL-1, IL-6, and TNF-α, blocking receptor-mediated reactions in target cells and potentiating the initial mechanisms of functional deficiency of iron [7,8]. It seems that high erythrodieresis is not only the initial mechanism of anemia, but is also a factor modulating the erythron kinetics. The 2-fold decrease in normocyte production under these conditions reduces iron utilization during the night hours, leading to leveling of sideremia circadian rhythm.

Hence, adaptive strain of terminal erythropoiesis promotes correction of LPO and retention of the basic parameters of spatial and temporal organization of erythropoiesis during remission. However, high plasticity of adaptive reactions is sensitive to iron deficiency developing as a result of erythrophagocytosis stimulation. The increasing volume of short-lived microcyte population and progressive functional deficiency of iron are the leading factors in the pathogenesis of anemia at the stage of CPN exacerbation.

TABLE 2. Rhythmometric Parameters of Iron Metabolism and MDA in Girls with CPN ($M\pm m$)

Group	Parameter	Mesor	Amplitude	Acrophase (95% confidence interval)
Healthy girls	Serum iron, μmol/liter	13.4±1.1	4.5±1.2	12.40 (11.15; 14.10)
(health group 1)	TSC, %	36.3±1.7	9.8±2.1	13.15 (11.25; 14.37)
	Serum ferritin, ng/ml	135.9±5.7	48.3±4.7	22.15 (21.05; 23.17)
	MDA, μmol/liter	25.8±1.3	2.2±0.4	01.41 (00.51; 02.53)
CPN remission	Serum iron, μmol/liter	13.0±1.9	3.1±1.7	No rhythm
	TSC, %	30.2±3.1	5.8±3.2	No rhythm
	Serum ferritin, ng/ml	205.9±9.7*	45.6±5.7	19.32 (18.05; 22.17)
	MDA, μmol/liter	32.7±1.1*	2.9±0.4	12.41 (10.51; 14.53)
CPN exacerbation	Serum iron, μmol/liter	10.3±2.9	2.5±1.8	No rhythm
	TSC, %	23.6±3.7	4.5±3.0	No rhythm
	Serum ferritin, ng/ml	312.5±19.5*	56.2±8.7	No rhythm
	MDA, μmol/liter	38.9±1.3*	4.2±1.4	No rhythm

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