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THE ROLE OF MELATONIN DEFICIENCY IN SYSTEMIC INFLAMMATION IN PATIENTS WITH TERMINAL RENAL FAILURE TREATED WITH HEMODIALYSIS

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Abstract

The aim of the study. Make analyze of the status of systemic inflammation in patients with chronic kidney disease (CKD) stage 5 treated with hemodialysis (HD) by assessing the level of C-reactive protein (CRP) and ferritin and to determine the relationship between disorders of melatonin-forming function of the epiphysis (MFE) and markers inflammation of CKD.

Materials and methods. 130 patients with stage 5 CKD (50% of men) aged 58.5 years were examined [43; 66] on permanent hemodialysis treatment. Controls consisted of 20 healthy individuals of equal age and sex. All surveyed performed the determination of the level of melatonin (MT) in saliva during the day and night. Depending on the violation of MFE patients are divided into two groups: group I - 110 patients with impaired MFE, group II - 20 patients with normal MFE. All patients performed clinical laboratory tests. The levels of ferritin, CRP, measured blood pressure (BP) are determined.

Results. We found high incidence of MFE disorders and their association with inflammatory activity in examined patients. Analysis of the data shows that patients with CKD stage 5 treated with HD, compared with the control group, higher levels of CRP, ferritin (17 [8; 23] vs 5 [3; 6,5] mg / ml, 311,4 [172.6; 505.4] vs. 73.3 [62.85; 105.9] ng / ml, both p <0.001). Greater activity of inflammation by the value of CRP is observed in patients with disorders of MFE (18 [12; 24] vs. 5.5 [4,5; 8] mg / ml). The level of CRP increases in the case of increased SBP (r = 0.389, p <0.05), DBP (r = 0.282, p <0.05), duration of hypertension (r = 0.278, p <0.05), HD (r = 0,257, p <0,05) and at the decrease of Hb (r = - 0,203, p <0,05), albuminemia (r = - 0,338, p <0,05). There were negative correlations between daytime and nighttime CRP and MT (r = - 0.267 and r = - 0.518, both p <0.05). We established positive associations of ferritin level with CRP levels (r = 0.614, p <0.05) and negative with MT level during the day (r = - 0.261, p <0.05) and at night (r = - 0.461, p <0,01). Positive associations of albuminemia with MT levels during day and night were identified (r = 0.231 and r = 0.303, both p <0.05).

Conclusions. There is a frequent violation of MFE in the form of MT deficiency and moderate activity of systemic inflammation, which is accompanied by increased ferritin, CRP and decreased levels of albuminemia in patients with stage 5 CKD undergoing hemodialysis treatment. The magnitudes of direct and indirect inflammatory markers are significantly increased in patients with NTF in the event of an MFE disorder, indicating an association between epiphysis dysfunction and inflammatory activity. In patients receiving HD therapy, the severity of inflammation is determined by the duration of NRT, the length and severity of hypertension, the severity of anemia, the depth of disorders of circadian rhythms MFE.

Keywords: chronic kidney disease, hemodialysis, melatonin, inflammation, ferritin, C-reactive protein, albumin.

Introduction. Chronic kidney disease (CKD) is an important problem of modern medicine, both in Ukraine and in the world. The number of patients undergoing renal replacement therapy (RRT) has increased faster than the world's population during recent years [6].

The combination of chronic inflammation syndrome with oxidative stress is more common and becomes more pronounced with the development of the terminal stage in patients with CKD. The increase in the activity of inflammation in patients with RRT may be

associated with a decrease in the clearance of anti-inflammatory cytokines, the development of endotoxemia and a decrease in the level of antioxidants [1, 4, 8].

The development of oxidative stress occurs under conditions of excessive free radical formation and it's closely associated with atherogenesis, endothelial dysfunction formation, and inflammation [10, 12, 13, 14]. This pathological phenomenon causes an increase the risk of cardiovascular complications and mortality [16].

Melatonin (MT) is an epiphysis hormone that exhibits more potent antioxidant effects than beta-carotene, selenium or vitamins C and E, has a special place in the range of antioxidants [11]. MT is a strong endogenous immunomodulator with anti-inflammatory properties [5, 15]. MT exhibits both immunological and non-immunological protective properties with the reduction of free radical formation in experimental sepsis modeling. It's ability to inhibit the activation of inflammatory cells by reducing myeloperoxidase activity has been demonstrated [7, 9].

Other work has shown some inhibition of the formation of proinflammatory cytokines and decrease activated macrophages and T-helper cells during repeated using of MT low doses for animals. MT has immunomodulatory properties, which is realized through specific receptors of MT1, MT2 and MT3 lymphoid organs and cellular elements of blood [2]. MT has a wide range of properties, but it's importance in the development and progression of a number of diseases remains to be fully understood. Outside of researchers attention is the evaluation of disorders of melatonin-forming function of the epiphysis (MFE) in CKD, especially in patients with RRT due to the syndrome of chronic inflammation.

The aim of the study. Investigate the status of systemic inflammation in hemodialysis (HD) patients with stage 5 CKD by assessing CRP and ferritin levels and determine the association with MFE disorder.

Materials and methods. The study involved 130 patients with stage 5 CKD and HD treatment in the conditions of the municipal non-profit enterprise "Kyiv City Center of Nephrology and Dialysis". There were 65 (50% among them) men average age of 58.5 [43; 66]. The average duration of RRT treatment was 11 years [6; 13]. The control group consists of 20 healthy individuals.

The study did not include patients younger than 18 years, with a duration of RRT ≤ 3 months, with severe history of cerebral and coronary artery disease, chronic heart failure III-IV functional class (NYHA classification), hemoglobin level (Hb) <70 g/l, with acute infectious processes of any etiology, diagnosed in the last 3 months, cancer, kidney transplant in history, with acute and chronic liver failure, refusal of the patient to participate in the study.

Mandatory methods of examination of patients included standard laboratory tests (general clinical and biochemical). Levels of ferritin, CRP and day and night levels of MT were determined for the screening of inflammation. Ferritin and CRP levels were determined on a Vitalab Flexor Junior analyzer. The values of ferritin 200-500 mcg / l, CRP <10 mg/l were considered as the reference.

The concentration of MT was determined by enzyme immunoassay using the Human MS (Melatonin Sulfate) ELISA Kit, Elabscience. Fence was carried out during the day and night, in the spring and summer, with a minimum illumination of 30 lx. Non-stimulated saliva was used and collected into a 1 ml Epindorf capsule and stored frozen at -20° C.

Office blood pressure (BP) measurements were performed before, during and after the HD session with the analysis of systolic BP (SBP), diastolic BP (DBP), pulse BP (PBP). For the target blood pressure level, an average blood pressure of ≤135/85 mm hg was taken.

The principles of bioethics and legal norms and requirements for biomedical research have been observed in our work. The research protocol was approved by the Commission for Bioethical Expertise and Ethics of Scientific Research at the O.O. Bogomolets National Medical University (Minutes №112 of May 31, 2018).

Demographic data and clinical characteristics of patients included in the study are presented in table 1.

Table 1

Clinical and demographic characteristics of patients with CKD treated with HD

Indicators	**Patients (n=130)**
BMI, kg / m2	21,3 [20,1; 22,3]
Waist volume, cm	88 [79; 92]
Office average SBP, mm Hg	150 [140; 160]
Office average DBP, mm Hg	90 [80; 92]
Hemoglobin, g / l	85 [77; 92]
Saturation of transferrin,%	32 [22,8; 36,3]
TC, mmol /l	4,31 [2,93; 5,62]
TG, mmol / l	1,52 [1,24; 1,77]
Uric acid, mmol / l	399 [372; 428]
iPTH (pg / ml)	530 [313; 614]
P (mmol / l)	1,88 [1,55; 2,03]
Ca^{2+} (mmol / l)	2,12 [1,98; 2,25]

Abbreviation: BMI - body mass index, SBP- systolic blood pressure, DBP- diastolic blood pressure, P – phosphorus, Ca2+ – calcium, iPTH - intact parathormone

Statistical results were processed using Microsoft Office Excel 2010 and IBM Statistics Spss 22. Student's test was used to compare normally distributed data, and non-parametric (U-test) Mann-Whitney was used for inconsistency of the law of normal distribution. Correlation was determined by Pearson's (r) and Spearman's methods depending on the distribution of indicators.

Results and discussion. Data analysis showed a statistically significant difference between the values of inflammation, Hb and albumin levels in patients with stage 5 CKD and control. Thus, the CRP level in patients with CKD 5 DG stage is 70.6% ($p < 0.001$) higher than the result obtained in almost healthy subjects. The level of ferritin in patients with RRT exceeded a similar indicator of the control group by 76.5% ($p < 0.001$).

The level of Hb in patients with HD was significantly lower than the result of the control group by 36.6% ($p < 0.001$). The albumin values of patients with stage 5 CKD were 12.5% ($p < 0.001$) lower than the values obtained for healthy individuals. The results obtained indicate the determined manifestations of inflammation in patients with HD, which is shown in table 2.

Table 2

Indicators characterizing the activity of inflammation in the study groups

Indicator	HD patients, n = 130	Control group, n = 20	p
CRP (g / l)	17 [8; 23]	5 [3; 6,5]	<0,001
Ferritin, ng / ml	311,4 [172,6; 505,4]	73,3 [62,85; 105,9]	<0,001
Hemoglobin, g / l	85 [77; 92]	134 [129; 136]	<0,001
Albumin (g / l)	35 [32; 37]	40 [38,5; 42,5]	<0,001

MT deficiency was detected in the majority of patients with CKD 5 HD stage (84.6%). Because of the epiphysis condition, patients with CKD 5 HD stage were divided into two groups: the main group included patients with impaired MFE (n=110) and the comparison group included patients with normal functioning of the epiphysis (n=20) [9].

Further comparative analysis of the groups of patients revealed a more pronounced degree of inflammatory activity in patients with impaired MFE. The ferritin index in the main group is 21.5% higher than the result of the control group, which is presented in table 3.

Patients with impaired MFE had statistically significantly higher CRP scores compared to patients with preserved MFE - 69.4% ($p < 0.001$) and lower albumin level by 6% ($p = 0.01$). It is known that the progression of CKD is accompanied by an accumulation of proinflammatory cytokines and a decrease of albumin level [3]. The results of our work confirm that the activity of inflammation is more pronounced in patients with epiphysis dysfunction.

Table 3

Comparative characteristics of inflammation in patients in the main group and patients with preserved MFE

Indicator	Control group, n = 20	Main group, n =110	p
CRP (g / l)	5,5 [4,5; 8]	18 [12; 24]	<0,001
Ferritin, ng / ml	287,55 [192; 342,5]	366,5 [167; 539,2]	0,2
Hemoglobin, g / l	85,5 [80,5; 94]	85 [76; 92]	0,2
Albumin (g / l)	36 [35; 37]	34 [32; 37]	0,01

The highest levels of CRP were determined in patients with hypertensive nephropathy, and the lowest in patients with polycystic kidney disease during the analysis of inflammation in the main group patients, depending on the etiological factor that led to the development of CKD. The highest level of ferritin was recorded in patients with urolithiasis, and the lowest with gouty nephropathy. The lowest level of albumin was determined in patients with hypertensive nephropathy, patients with polycystic kidney disease had the highest rate, which is presented in table 4.

Therefore, the highest inflammatory activity among patients with CKD and HD is inherent in individuals who have hypertensive nephropathy as a cause of RRT. It is worth noting that the correlation between the concentration of proinflammatory cytokines and cardiovascular mortality in patients with RRT is related to their impact on early development and progression of atherosclerosis [10].

Table 4

Comparative characteristic of inflammation in the main group patients depending on nosology

Indicators	Hypertensive nephropathy, n= 38	Glomerulonephritis, n= 37	Gouty nephropathy, n= 16	Polycystic disease, n= 5	Urolithiasis, n= 5	Tubulointerstitial nephritis, n= 9
CRP (g/l)	19 [14; 26]	17 [9; 24]	17 [7,5; 19] *	15 [13; 19]	19 [18; 24]	18 [18; 22]
Ferritin, ng/ml	415,3 [275,5; 604,7]	256,8 [138,2; 433,4]	229,4 [78,8; 409,9] *	478,5 [282; 594]	505,4 [317; 521]	432,8 [271; 590,3]
Hemoglobin, g/l	83,5 [75; 89]	87 [78; 92] #	77,5 [74; 87,5]	89 [79; 92]	75 [74; 87]	90 [83; 94] #
Albumin (g/l)	33 [30; 35]	35 [33; 38]	35 [34; 38,5] *	36 [33; 37]	35 [34; 35]	33 [31; 36]

Notes:
1. * - p <0.05 compared with hypertensive nephropathy patients;
2. # - p <0.05 compared with gouty nephropathy patients.

We performed a comparative analysis of the severity of inflammation in patients with stage 5 CKD depending on the duration of treatment of HD, which demonstrates the most significant increase in its activity in patients with the greatest "experience" of RRT. Patients who used HD during 10-15 years have the highest level of CRP, ferritin, and the lowest level of Hb and albumin, possibly related to epiphysis dysfunction, development of oxidative stress, and increased synthesis of proinflammatory cytokines [1].

Table 4

The activity of inflammation in the main group patients depending on the HD duration

Indicator	1 – 5 years, (n=24)	5 -10 years, (n=20)	10-15 years, (n=66)
CRP (g/l)	15 [7; 21,5]	17,5 [10,5; 21]	19 [14; 24]
Ferritin, ng/ml	199,9 [87,9; 398,25]	338 [198,7; 466,5]	413,05 [189,3; 601,1] *
Hemoglobin, g/l	89 [79,5; 92]	87 [77,5; 93,5]	82,5 [75; 89] *
Albumin (g/l)	35 [33,25; 38]	34 [31,5; 36,5]	34 [31; 36]

Note. * - p <0,05 compared with patients of group I.

There is some dependency between MT and AP levels, between proinflammatory cytokines and cardiovascular complications in patients with CKD. The interesting fact is the inverse correlations of equal strength between the levels of SBP and DBP and the level of MT in the daytime (r = -0.66, r = -0.61, both p <0.001) and the night period were established (r = -0.7, r = -0.66, both p <0.001) during the study. It was found that the levels of day and night MT have an inverse corelation with the duration of hypertension (r = - 0.61 and r = -0.66, both p <0.05), which may indicate a higher risk of cardiovascular complications in patients with impaired MFE [9].

Thus, a comparative analysis of the severity of inflammation in patients of the main group, depending on the duration of hypertension shows their deepest disorders in patients with the highest duration of hypertension, which is presented in table 5. The values of acute-phase index of inflammation of CRP in patients with the greatest "experience" of hypertension (more than 15 years) higher in comparison with the value of this indicator in patients with a duration of hypertension from 5 to 10 years by 35.7% (p <0,05). A similar prevalence is observed in the analysis of indicators of ferritin.

Table 5

Comparative characteristic of inflammation in the main group patients depending on the duration of hypertension

Indicators	I group (1-5 years), (n=10)	II group (5-10 years), (n=27)	III group (10-15 years), (n=32)	IV group (>15 years), (n=41)
CRP (g/l)	18 [11; 24]	14 [6; 18]	19 [14; 23] #	19 [14; 26] #
Ferritin, ng/ml	188,75 [87,4; 405]	206,8 [86,2; 391,5]	398,65 [169,1;525,6] #	435,2 [271; 603,2] *#
Hemoglobin, g/l	85 [78; 89]	90 [81; 94]	81,5 [74,5; 89,5] #	82 [75; 88] #
Albumin (g/l)	34,5 [33; 39]	35 [33; 37]	34,5 [31; 37]	34 [31; 36]

Notes:
1. * - p <0,05 compared with group I;
2. # - p <0,05 compared with group II.

The analysis of direct and indirect indicators characterizing inflammation depending on the circadian rhythms of MT made in patients with stage 5 CKD treated with HD. Thus, in patients with the lowest levels of daytime MT in saliva, the highest values of ferritin and the lowest levels of albumin are determined, which is presented in table 6.

Table 6

Comparative characteristic of inflammation of the main group patients depending on the level of daytime MT in saliva

Indicators	I group (2,6-3,9 pg/ml, (n=26))	II group (1,3 -2,6 pg/ml, (n=68))	III group (<1,3, pg/ml, (n=16))
CRP (g/l)	16 [8;19]	18 [13,5; 24]	18,5 [12,5; 23,5]
Ferritin, ng/ml	207,5 [81,5; 372]	414,8 [170,3; 592,15] *	344 [263,9; 492,1] *
Hemoglobin, g/l	88 [81; 92]	82,5 [75; 91]	82 [75; 90,5]
Albumin (g/l)	35 [33,5; 38]	34 [31; 37]	33,5 [30,5; 35] *

Note. * - p <0,05 compared with group I.

It should be noted that patients with the lowest level of nocturnal MT was characterized by the highest ferritin values and the lowest albumin values and the Hb level, which is presented in table 7.

Table 7

Comparative characteristic of inflammation of the main group patients depending on the night level of MT in saliva

Indicators	I group (39 – 49,1 pg/ml (n=9))	II group (26 – 39 pg/ml (n=27))	III group (12,6 - 25 pg/ml (n=74))
CRP (g/l)	15 [8; 18]	17 [7; 21]	18,5 [13; 24]
Ferritin, ng/ml	362 [198,8; 405]	201 [81,5; 400,3]	413,05 [202; 590,3] *
Hemoglobin, g/l	87 [85; 92]	89 [79; 94]	82 [75; 90] *
Albumin (g/l)	34 [32; 35]	35 [33; 38]	34 [31; 36] *

Note. * - p <0.05 compared with group II.

Correlation analysis determines the positive depending of the average strength of ferritin with CRP (r = 0,614, p <0,05), the positive correlation with the level of SBP (r = 0,281, p <0,05), body mass index (r = 0.321, p <0.05), more closely with the duration of treatment with HD (r = 0.325, p <0.05) and AH (r = 0.365, p <0.05). The weak negative correlation between ferritin and MT level is determined both in the daytime (r = -0.261, p <0.05) and in the night period, which is presented in Fig. 1.

Therefore, the magnitude of the indicator that indirectly reflects the activity of inflammation increases with increasing of AP and the duration of hypertension, the degree of obesity, and the length of RRT in the event of MFE disruption.

A nephrologist should remember the association of ferritin with chronic inflammatory syndrome in patients with CKD, which is often associated with a decrease in nutritional status and existing subclinical infections.

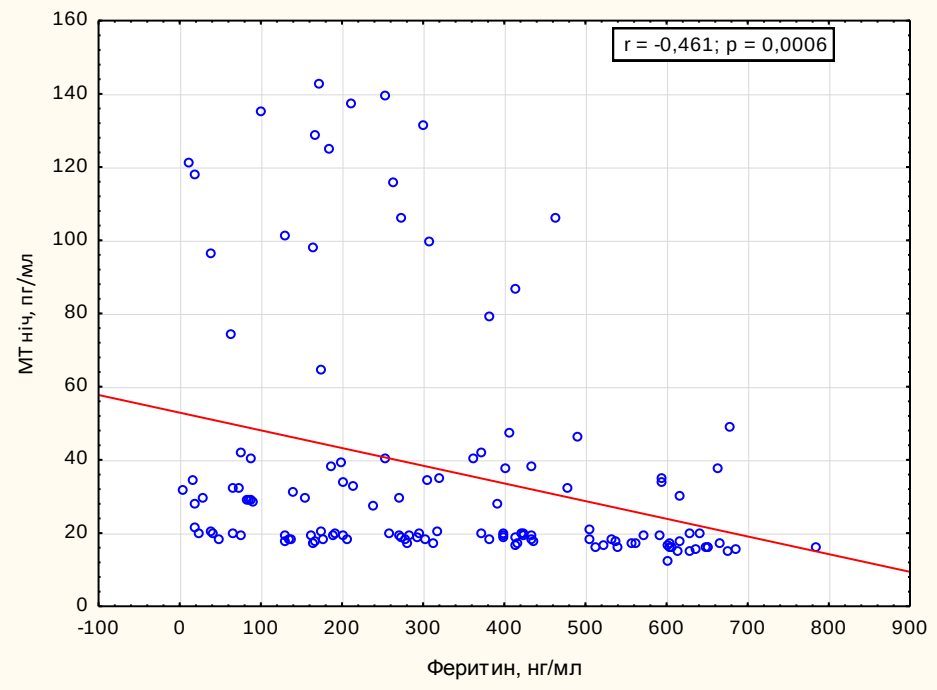

Fig. 1. Correlation between night MT and ferritin.

In patients of the main group correlation analysis shows that the value of acute-phase inflammation - CRP increases in the case of increased SBP (r = 0,389, p <0,05), DBP (r = 0,282, p <0,05), the duration of hypertension (r = 0.278, p <0.05) and HD (r = 0.257, p <0.05). At the same time, the CRP level increases with

the decrease of Hb (r = - 0,203, p <0,05), albuminemia (r = - 0,338, p <0,05), MT in the daytime (r = - 0,267, p <0,05)) and the night period shown in Fig. 2.

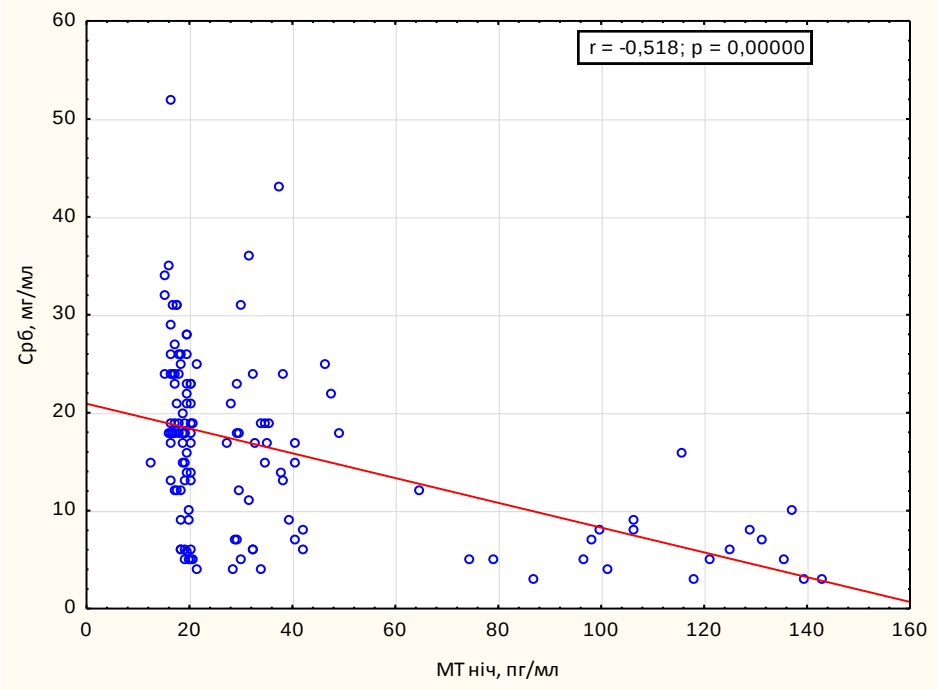

Fig. 2. Correlation between night MT and CRP.

It was found that the level of albuminemia decreased with increasing ferritin (r = - 0,352, p <0,05), the duration of hypertension (r = - 0,219, p <0,05), the duration of hemodialysis (r = - 0,183, p <0,05)), the level of SBP (r = - 0,181, p <0,05) and DBP (r = - 0,193, p <0,05). However, the level of albuminemia is directly related to the level of MT per day (r = 0.231, p <0.05) and more closely during the night period, which is presented in Fig. 3.

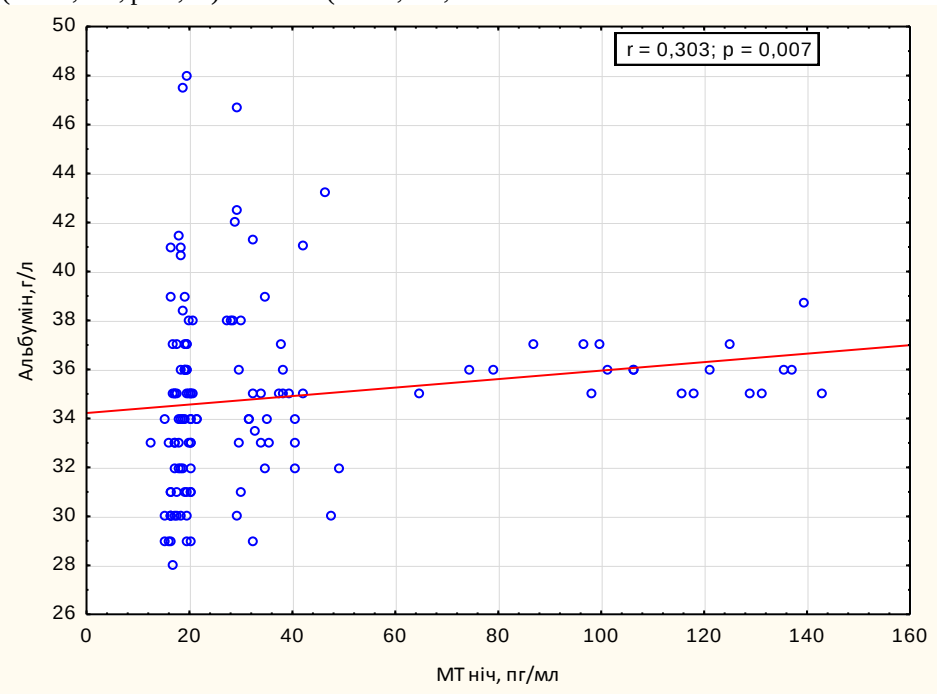

Fig. 3. Correlation between nightly MT and albumin levels

Assessing the correlation of inflammatory activity with, it can be assumed that normalization of MT may be accompanied by a reduction in the incidence of inflammation in patients undergoing HD treatment and requires more thorough further study.

Conclusions. There is a frequent violation of MFE in the form of MT deficiency and moderate activity of systemic inflammation, accompanied by increased ferritin, CRP and decreased albuminemia in patients with stage 5 CKD undergoing hemodialysis. The magnitudes of direct and indirect inflammatory markers are

significantly increased in patients with end-stage renal insufficiency in the case of MFD disorders, which may indicate a close correlation between epiphysis dysfunction and inflammatory activity. The severity of inflammation is determined by the duration of RRT, the length and severity of hypertension, the severity of anemia, the depth of disorders of circadian rhythms of MFE in patients receiving hemodialysis therapy.

Prospects for further research. Changes in the severity of inflammation in patients with CKD stage 5 and HD treated with melatonin are a subject of further research and will be presented in subsequent reports.

Conflict of interests. The authors declare no conflict of interest.

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OXIDATIVE-ANTIOXIDANT STRESS AS A MARKER OF IMDALANCE OF ORAL FLUID HOMEOSTASIS IN PATIENTS WITH GENERALIZED PARODONTAL DISEASES AND ANOREXIA NERVOSA

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Abstract

Objective. To study the balance of fatty acid spectrum of oral lipids as a marker of oxidant - antioxidant stress in patients with generalized parodontitis with anorexia nervosa.

Materials and methods. The study metabolic disorders of fatty acids of the oral fluid was conducted in 28 patients with GP, primary-I, I-II degree, chronic course associated with anorexia nervosa, (average age 26 ± 3.8)