Predictive criteria of oxidative stress in struvite cystolithiasis in cats taking into account intercorrelations of iron metabolism and redox regulation of blood cells

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Abstract. The existing diagnostic protocols for feline cystolithiasis are extremely limited because they do not take into account the nature of the relationship between iron metabolism markers and the level of oxidative stress. Correlation analysis of this relationship will help find predictive serum biomarkers that can be used to stratify the risk of oxidative stress and predict subsequent metabolic disorders in feline struvite cystolithiasis. The aim of the experiment was to conduct exploratory studies of predictive factors of cellular redox regulation disorders in feline struvite cystolithiasis. The object of the study was cats with severe manifestations of struvite cystolithiasis (n=30). Standard methods of hematological and biochemical analysis of blood serum and statistical methods were used in the study. The analysis of the relationship between the studied parameters of blood cells in experimental cats indicated the presence of a direct correlative relationship between most of the signs. Predictive risk stratification of oxidative stress using RHE, MCV, MCHC and serum iron (Fe+2) levels will improve the prediction of subsequent metabolic abnormalities in feline struvite cystolithiasis.

1 Introduction

The development of inflammatory processes in the urogenital tract in animals is closely associated with the processes of activation of lipid peroxidation [1 - 4], and an increase in the duration of the course and the development of metabolic disorders in struvite-type cystolithiasis in cats further strengthens the formed cause-and-effect relationship [5 - 8].

Maintenance of oxidative metabolism of body cells is carried out through the production of activated oxygen metabolites and the functioning of the endogenous antioxidant potential, and the development of most pathological processes and conditions leads to a violation of this balance [9 - 10]. The state of redox regulation of cells is an important risk factor for the development and progression of many diseases.

Existing diagnostic protocols for cystolithiasis in cats are extremely limited, since they do not take into account the role of oxidative stress in the initiation and subsequent progression of morphofunctional disorders in the liver-kidney axis [11 - 13]. Analysis of

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the nature of the relationship between markers of cellular redox regulation and iron metabolism will allow us to find predictive serum biomarkers that can be used to stratify the risk of oxidative stress and predict subsequent metabolic disorders in struvite cystolithiasis in cats. The aim of the presented experiment was to conduct exploratory studies of predictive factors of oxidative degradation of lipids in struvite cystolithiasis in cats, taking into account the nature of the relationship between iron homeostasis markers and redox metabolism of blood cells.

2 Methods and equipment

In the framework of exploratory studies, a selection of thirty males of the domestic cat species (Felis catus) of the crossbreed breed with pronounced manifestation of struvite cystolithiasis at the age of 8-10 years was carried out using the method of analogous pairs. Patients were combined into two experimental groups - experimental (n=15) and control (n=15).

The criteria for inclusion of animals in the experimental groups were: initial stage of anemic syndrome (RBC – 6.10±0.50×10^{12}/l and 5.73±0.83×10^{12}/l; HGB – 83.00±4.90 g/l and 88.00±6.01 g/l; HCT – 0.237±0.005 % and 0.241±0.007 %; MCV – 38.90±2.18 fl and 42.10±2.55 fl; MCH – 13.70±1.97 pg and 15.30±2.05 pg; MCHC - 350.00±9.10 g/l and 365.00±10.62 g/l) against the background of iron deficiency (Fe^{+2} – 11.93±0.50 µmol/l and Fe+2 – 12.00±0.68 µmol/l) and normal proliferative activity of the bone marrow (RET# - 17.10±2.06×10^{9}/l and 40.10±4.37×10^{9}/l; RHE - 16.40±1.03 pg and 16.00±1.14 pg); decreased activity of the enzymatic link of antioxidant protection (SOD - 207.63±5.30 U/ml and 170.80±7.06 U/ml; GTP - 6451.09±19.10 U/g Hb and 6569.80±24.89 U/g Hb) and general antioxidant status (TAS - 1.24±0.05 mmol/l and 1.30±0.03 mmol/l), increased activity of lipid peroxidation (MDA - 1.69±0.17 nmol/ml and 1.55±0.20 nmol/ml; 8-OHdG - 0.22±0.03 ng/ml and 0.18±0.05 ng/ml).

In order to establish the nature of the correlation between iron metabolism markers and the level of redox regulation of blood cells in cats with struvite cystolithiasis, hematological studies were performed using the standard flow impedance method, laser scattering, colorimetric method and SF Cube cell analysis technology, as well as biochemical studies using standard enzyme immunoassay methods, and correlation (Spearman correlation coefficient - rs) and correlation-regression analysis (Pearson coefficient - r-Pearson, determination coefficient - r^2, covariance - cov (X, Y)).

3 Results

The conducted analysis of the relationship between the studied parameters of blood cells in experimental cats indicated the presence of a direct correlation between most of the signs, but the level of superoxide dismutase (SOD) activity was inversely related to the mean corpuscular hemoglobin concentration (MCHC) in both groups, and a negative correlation was found between the level of glutathione peroxidase (GTP) activity and the hematocrit index (HCT), and between 8-OH-deoxyguanosine (8-OHdG) and hemoglobin equivalent (RHE) in the control group (Table 1). The greatest number of correlations were characteristic of the level of superoxide dismutase and glutathione peroxidase activity, as well as the 8-OH-deoxyguanosine index. The level of SOD activity had a reliable correlation with the value of hemoglobin (SOD-HGB), hematocrit (SOD-HCT), mean corpuscular hemoglobin (SOD-MCH), and hemoglobin equivalent (SOD-RHE). The activity of the redox status of cells was characterized by the presence of a similar intercorrelation pattern in the level of GTP activity and mean corpuscular volume (GTP-

MCV), mean corpuscular hemoglobin concentration (GTP-MCHC), and absolute reticulocyte count (GTP-RET#). The same direction of correlation was noted between 8-OHdG and hemoglobin concentration (8-OHdG-HGB), hematocrit (8-OHdG-HCT), mean corpuscular volume (8-OHdG-MCV), and serum iron level (8-OHdG-Fe^{+2}).

Table 1. Spearman correlation coefficient (rs) between markers of iron metabolism and redox regulation of blood cells in cats with struvite cystolithiasis.

Indicators	Group of animals (n = 30)				
	SOD (X)	TAS (X)	GTP (X)	MDA (X)	8-OHdG (X)
Experimental group (n=15)					
RBC (Y)	0.482	0.484	0.507	0.482	0.402
HGB (Y)	0.632**	0.192	0.035	0.109	0.034
HCT (Y)	0.388	0.459	0.028	0.729**	0.200
MCV (Y)	0.071	0.554*	0.607**	0.259	0.690**
MCH (Y)	0.559*	0.336	0.123	0.370	0.059
MCHC (Y)	-0.082	0.378	0.707**	0.134	0.469
RET# (Y)	0.089	0.218	0.432	0.361	0.582*
RHE (Y)	0.581*	0.296	0.134	0.529*	0.277
Fe^{+2} (Y)	0.157	0.430	0.417	0.109	0.351
Control group (n=15)					
RBC (Y)	0.343	0.273	0.457	0.381	0.330
HGB (Y)	0.019	0.220	0.389	0.385	0.583*
HCT (Y)	0.607**	0.001	-0.143	0.232	0.727**
MCV (Y)	0.110	0.419	0.539*	0.047	0.404
MCH (Y)	0.362	0.403	0.396	0.431	0.440
MCHC (Y)	-0.055	0.276	0.796***	0.317	0.225
RET# (Y)	0.493	0.515*	0.614*	0.036	0.086
RHE (Y)	0.527*	0.725**	0.335	0.759***	-0.009
Fe^{+2} (Y)	0.212	0.043	0.414	0.005	0.679**

Note: * - P< 0,05; ** - P< 0,01; *** - P< 0,001 compared to the critical value of the Spearman criterion for a given number of degrees of freedom

Chaddock's closeness of connection scale:

- weak
- moderate
- noticeable
- high
- strong

High correlation strength was revealed in cats between the following clinical and laboratory markers of oxidative stress and iron metabolism indices: GTP - MCHC, MDA - HCT in the experimental group, and TAS - RHE, GTP - MCHC, MDA - RHE, 8-OHdG - HCT in the control group. At the same time, the intensity of intercorrelation in the experimental group exceeded the similar nature of the relationship in the control group by 214.22% between the level of MDA and HCT, and in the control group the difference in the Spearman correlation coefficient for a number of features was higher by 144.93% (between TAS and RHE), 12.59% (between GTP and MCHC), 43.47% (between MDA and RHE), by 263.50% (between 8-OHdG and HCT).

Depletion of endogenous antioxidant capacity was associated in a co-directional manner with feline anemia syndrome, with a stronger association with superoxide dismutase (SOD) activity in subjects with lower HGB and MCH, and higher HCT and RHE. A stronger

association with GTP activity was seen in subjects with higher MCHC and RET#, and lower MCV. A higher absolute reticulocyte count (RET# - 40.10±4.37×10^9/l (control group)) and a lower mean corpuscular volume (MCV - 38.90±2.18 fl (experimental group)), hemoglobin equivalent (RHE - 16.00±1.14 pg (control group)) are more closely associated with the level of total antioxidant status (TAS).

The highest association with malondialdehyde (MDA) was observed at a lower value of hematocrit (HCT – 0.237±0.005% (experimental group)) and hemoglobin equivalent (RHE - 16.00±1.14 pg (control group)), and with 8-OH-deoxyguanosine (8-OHdG) - at a higher value of hemoglobin (HGB – 83.00±4.90 g/l (control group)), hematocrit (HCT – 0.241±0.007% (control group)), iron (Fe^{+2} – 12.00±0.68 µmol/l (control group)) and a lower level of the mean corpuscular volume (MCV – 38.90±2.18 fl (experimental group)), absolute reticulocyte count (RET# - 17.10±2.06×10^9/l (experimental group)) (Table 1).

The conducted correlation and regression analysis between the parameters reflecting the depletion of the endogenous antioxidant potential of cells in cats with struvite cystolithiasis indicated the presence of a strong positive relationship between TAS and RHE (control group), while the change in hemoglobin equivalent is explained by a change in the total antioxidant status by 52.20% (r^2 = 0.522), between GTP and MCHC (experimental and control groups) with a determination coefficient from 0.528 to 0.657 (Table 2).

Table 2. Pearson correlation coefficient (r-Pearson) between parameters reflecting depletion of endogenous antioxidant potential of cells in cats with struvite cystolithiasis.

Indicators	Group of animals (n = 30)								
	SOD (X)			TAS (X)			GTP (X)		
	r-Pearson	r^2	cov (X,Y)	r-Pearson	r^2	cov (X,Y)	r-Pearson	r^2	cov (X,Y)
Experimental group (n=15)									
HGB (Y)	0.507*	0.257	6.235	0.158	0.025	0.019	0.054	0.002	2.113
MCV (Y)	-0.025	0.0006	-0.167	0.585*	0.342	0.039	0.547*	0.299	11.587
MCH (Y)	0.538*	0.289	2.496	0.329	0.108	0.015	0.119	0.014	1.764
MCHC (Y)	-0.037	0.001	-0.747	0.434	0.188	0.086	0.726**	0.528	45.721
RET# (Y)	0.175	0.030	0.704	0.385	0.148	0.015	0.542*	0.294	6.914
RHE (Y)	0.554*	0.307	1.426	0.412	0.169	0.010	0.103	0.010	0.848
Control group (n=15)									
HGB (Y)	-0.050	0.002	-0.968	0.142	0.020	0.016	0.290	0.084	22.587
MCV (Y)	0.179	0.032	1.174	0.412	0.170	0.016	0.536*	0.288	14.291
MCH (Y)	0.372	0.138	2.005	0.427	0.182	0.013	0.312	0.097	6.841
MCHC (Y)	-0.146	0.021	-5.097	0.281	0.079	0.059	0.810***	0.657	114.880
RET# (Y)	0.508*	0.258	6.986	0.457	0.208	0.037	0.562*	0.316	31.411
RHE (Y)	0.465	0.216	1.740	0.723**	0.522	0.016	0.255	0.065	3.885

Note: * - P< 0,05; ** - P< 0,01; *** - P< 0,001 compared to the critical value of the Pearson criterion for a given number of degrees of freedom

Chaddock's Tightness of Connection Scale:

- weak
- average
- strong

The nature of the established covariance between the studied characteristics indicated a unidirectional change in most characteristics, with the exception of cov (SOD, MCV) and cov (SOD, MCHC) in the experimental group, and cov (SOD, HGB) and cov (SOD, MCHC) in the control group, where the cause-and-effect relationship was characterized by an increase in one and a decrease in the other characteristic.

Also, a strong positive correlation was recorded between MDA and HCT (experimental group), with the determination coefficient explaining 49.30% of the change in the studied traits, between MDA and RHE (r^2 = 0.536) in the control group, between 8-OHdG and MCV (experimental group), between 8-OHdG and HCT (control group), between 8-OHdG and Fe^{+2} with a variation share equal to 0.495, 0.529 and 0.552 for the indicators, respectively (Table 3).

Table 3. Pearson correlation coefficient (r-Pearson) between parameters reflecting the level of free radicals in the body (lipid peroxidation) in cats with struvite cystolithiasis.

Indicators	Group of animals (n = 30)					
	MDA (X)			8-OHdG (X)		
	r-Pearson	r^2	cov (X,Y)	r-Pearson	r^2	cov (X,Y)
Experimental group (n=15)						
HCT (Y)	0.702**	0.493	0.0002	0.214	0.046	0.00001
MCV (Y)	0.261	0.068	0.049	0.703**	0.495	0.028
RET# (Y)	0.449	0.201	0.051	0.676**	0.457	0.016
RHE (Y)	0.629**	0.396	0.046	0.392	0.153	0.006
Fe^{+2} (Y)	0.122	0.014	0.004	0.360	0.130	0.002
Control group (n=15)						
HCT (Y)	0.217	0.047	0.00001	0.727**	0.529	0.00008
MCV (Y)	0.111	0.012	0.002	0.410	0.168	0.016
RET# (Y)	-0.029	0.0008	-0.001	0.059	0.003	0.005
RHE (Y)	0.732***	0.536	0.008	-0.050	0.002	-0.001
Fe^{+2} (Y)	-0.022	0.0005	-0.0001	0.743***	0.552	0.009

Note: * - P< 0,05; ** - P< 0,01; *** - P< 0,001 compared to the critical value of the Pearson criterion for a given number of degrees of freedom
Chaddock's Tightness of Connection Scale:
- weak
- average
- strong

Correlations between the occurrence of a possible cause of lipid peroxidation in the body of cats with struvite cystolithiasis indicated a parallel conjugation between most of the signs, and cov (MDA, RET#), cov (MDA, Fe^{+2}) and cov (8-OHdG, RHE) in the control group went in different directions (Table 3).

Based on the above, a decrease in the hemoglobin equivalent (RHE) level, which has the greatest conjugation with SOD and TAS, as well as a decrease in the mean corpuscular volume (MCV) and an increase in the mean corpuscular hemoglobin concentration (MCHC), correlating with GTP, can be considered as a reliable predictor criterion for a decrease in the level of endogenous antioxidant potential of blood cells with struvite cystolithiasis in cats. Predictive biomarkers of lipid peroxidation may include a decrease in the hemoglobin equivalent (RHE) level, which shows the closest correlation with MDA, a decrease in the mean corpuscular volume (MCV) and serum iron (Fe^{+2}) levels, which have the most stable relationships with 8-OHdG.

4 Discussion

The established nature of the relationship between the markers of iron metabolism and redox regulation of blood cells in experimental animals indicated a correlation between a higher hemoglobin equivalent (RHE ≥ 16.40±1.03 pg) and the level of superoxide dismutase (SOD) activity, and a lower hemoglobin equivalent (RHE ≤ 16.00±1.14 pg) - with the level of total antioxidant status (TAS), a close correlation was established between a higher level of the average concentration of hemoglobin in erythrocytes (MCHC ≥ 365.00±10.62 g/l), a lower value of the average erythrocyte volume (MCV ≤ 38.90±2.18 fl) and the level of glutathione peroxidase (GTP) activity. The highest association with malondialdehyde (MDA) was observed at a lower hemoglobin equivalent (RHE ≤ 16.00±1.14 pg), and with 8-OH-deoxyguanosine (8-OHdG) - at a higher iron (Fe^{+2} ≥ 12.00±0.68 μmol/l) and a lower mean corpuscular volume (MCV ≤ 38.90±2.18 fl). The

representative nature of the relationship between the studied iron metabolism markers and the level of cellular redox metabolism is determined by the change in the functional activity of hematopoietic tissue under the influence of oxidative degradation of lipids in struvite cystolithiasis in cats.

5 Conclusion

Thus, the use of changes in the hemoglobin equivalent (RHE) level, the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) as predictive criteria for the depletion of the endogenous antioxidant potential of the cell, hemoglobin equivalent (RHE), mean corpuscular volume (MCV) and iron level (Fe+2) - for oxidative stress risk stratification will improve the reliability of the prognosis of subsequent metabolic disorders that develop against the background of the inflammatory process in the urogenital tract in cats with struvite cystolithiasis. These markers will also allow for more efficient monitoring of therapeutic measures for struvite cystolithiasis in cats.

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