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CIRCADIAN RHYTHM OF METABOLIC RATES IN THE SALIVA OF PATIENTS WITH ARTERIAL HYPERTENSION AGAINST THE BACKGROUND OF TYPE 2 DIABETES MELLITUS

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Abstract

According to our hypothesis, one of the mechanisms in the development and progression of cardiovascular diseases may be metabolic changes that are inadequate with the functional responses of adaptation, indicating the disruption of the functional and metabolic continuum (FMC).

We suggested to study the daily rate of change in metabolism and hormonal regulation (morning vs. evening) in the saliva to diagnose the FMC disorders. In order to detect the FMC changes in patients with diabetes mellitus (DM2) complicated by arterial hypertension (AH),

we studied the parameters of FMC and endocrine regulation in the saliva in the morning and in the evening as compared to patients with diabetes mellitus.

Previously, we have shown that in compensated DM2, there are changes in the energy metabolism, induced by hormonal regulation. These changes can be controlled with daily saliva monitoring.

The study of the daily rhythm of in the saliva parameters makes it possible to conclude that in DM2, and especially in its progress, as evidenced by the accession of hypertension, there are disorders of FMC. One of its consequences is the development of oxidative stress, which is considered as one of the leading secondary mechanisms in the pathogenesis of DM2.

Key words: oxidative stress, metabolism, saliva, daily rhythms, diabetes mellitus, arterial hypertension.

In the XXI century, the steady increase in the prevalence of type 2 diabetes mellitus (DM2), as well as the frequency of its serious consequences, cause great concern to the world medical community. It is important that at the moment of diagnosis of DM2, the prevalence of complications that lead to a decrease in the quality of life, early disability and death is already high enough: 50% of patients already have coronary artery disease, 20% suffer from retinopathy and 20% have microalbuminuria. As is known, cardiovascular disease (CVD) is the main cause of death in 52% of diabetic patients [1].

In DM, CVD occurs 2-5 times more often than in people without this pathology. At the same time, the risk of developing conditions such as coronary heart disease (CHD), myocardial infarction (MI), arterial hypertension (AH), and acute cerebrovascular disease (ACVD) increases. Thus, 69% of patients with diabetes have dyslipidemia, 80% − hypertension, 50-75% − diastolic dysfunction, 12-22% − chronic heart failure (CHF) [1].

AH is a risk factor for the progression and formation of cardiovascular complications due to the development of pathological mechanisms, associated with damage and endothelial dysfunction of vessels, formation of insulin resistance (IR), activation of proinflammatory reactions, and disorders of the coagulation cascade, which leads to the prothrombotic state that is the basis of pathological processes responsible for the progression of atherosclerosis and increased cardiovascular risk [2, 3, 4]. The situation is complicated by the fact that among patients with hypertension, 50-75% have concomitant obesity. Association of these pathological states is a “vicious circle” wherein each component is interdependent relative to another, which contributes to the progression of these diseases [2, 5].

Numerous research works of recent years indicate the association of diseases such as atherosclerosis, coronary heart disease, hypertension and diabetes mellitus, with deterioration of endothelium dependent vasodilation, mediated by decreased endothelial production or bioavailability of nitric oxide (NO). Consequently, the issue of oxidative stress also has a definite contribution to the development of CVD. In oxidative stress, there is an increased formation of free radicals and a decrease in antioxidant activity, which disturbs the balance of oxidative-reducing reactions and leads to serious changes in cellular function, with subsequent damage to the cell structure. With the participation of free radicals, peroxide oxidation of lipids also increases, resulting in the imbalance between the production of vasoconstrictor, prothrombotic and proliferative factors, on the one hand, and vasodilating, angioprotective and antiproliferative factors, on the other hand. Thus, all this can play an important role in reducing the coronary reserve in patients with AH with intact coronary arteries [1].

Comorbidity of AH and DM2 is a serious problem due to an earlier development of lesion to target organs and subsequent cardiovascular catastrophes [6].

Hyperglycemia is a major metabolic symptom of DM2, but insufficiency of insulin secretion begins long before the diagnosis is made – even at the pre-diabetic glycemic level. The likelihood of the development of fatal and non-fatal cardiovascular complications increases significantly not only in apparent disease, but also at the stage of pre-diabetes [7]. Already at the stage of glucose tolerance disturbance (GTD), the incidence of lesion to coronary and cerebral arteries with atherosclerosis is significantly higher than in normal glycemia. IR is a key link in the pathogenesis of DM2, atherosclerosis, hypertension and other diseases. IR is an independent risk factor for the development of dyslipidemia, systemic inflammation and oxidative stress [8].

The published results of clinical and experimental studies indicate that IR causes disruption of physiological mechanisms of vasodilatation. The effect of insulin on the vascular wall endothelium is mediated by its own receptors and is realized through a multi-stage signaling system associated with an increase in the synthesis of nitric oxide (NO). In patients with hypertension and concomitant DM2, induced NO endothelium-dependent vasodilation is significantly reduced under the conditions of IR. It is believed that as a result of the activity of the cascade of proinflammatory cytokines, fat accumulation in adipocytes, liver, muscle, β-cells of the pancreas is observed simultaneously with increased lipolysis and the development of IR adipocytes [9]. The study on stratification of risk in patients with hypertension and diabetes mellitus conducted in Ukraine showed that almost half of patients

with DM2 and uncontrolled AH have a very high 4-year risk of cardiovascular complications characterized by high blood pressure and the presence of concomitant pathological changes. AH and DM2 are the components of metabolic syndrome (MS), and therefore they often occur against the background of obesity [9]. Data from epidemiological studies indicate that overwhelming majority of patients with diabetes mellitus are overweight or obese. Thus, in case of degree I obesity, the risk of diabetes mellitus increases by 2 times, degree II – 5 times, degree III – more than 10 times [10].

It has been established that the pathogenetic mechanisms, which predetermine the development of hypertension, IR and DM2, largely overlap and lead to disease progression and development of complications. In patients with hypertension and DM2, concomitant obesity is a factor in the progression of metabolic disorders, IR, activation of markers of immune inflammation and atherogenesis.

In its turn, visceral obesity contributes to the imbalance of adipocytokines production, which leads to a chain of pathophysiological disorders in the body. The imbalance of adipocytokines aggravates the course of DM2 and contributes to a worsening of carbohydrate metabolism. Thus, an integrated approach to the study of genetic predictors in the formation of combined AH and DM type 2, and directly IR, can help to select a more accurate therapeutic tactics for patients with AH and DM 2 [6].

Recently, much attention has been given to chronotherapy to increase the effectiveness of antihypertensive treatment, to normalize the daily profile of blood pressure in patients with night-time AP increase (“night-peakers”) or inadequate decrease (“non-dippers”). The reduction in the incidence of cardiovascular events and mortality for five years during the administration of hypotensive drugs before going to sleep [11, 31] has been shown, emphasizing the presence of vegetative regulation of blood pressure in diabetic patients with a lack of its adequate reduction in sleep [11].

According to our hypothesis, one of the mechanisms in the development and progression of cardiovascular diseases may be metabolic changes that are inadequate with the functional responses of adaptation, indicating a disruption of the functional and metabolic continuum (FMC) [12].

We suggested to study the daily rate of change in metabolism and hormonal regulation (morning vs. evening) in the saliva to diagnose the FMC disorders [13,14]. In order to detect the FMC changes in patients with diabetes mellitus (DM2) complicated by arterial hypertension (AH), we studied the parameters of FMC and endocrine regulation in the saliva in the morning and in the evening as compared to patients with diabetes mellitus.

The aim of the research

The purpose of this research was to examine the daily rhythm of FMC in patients suffering from AH against the background of DM2 according to the study of saliva.

Materials and methods of the research

The object of the research embraced unstimulated oral fluid (mixed saliva), which was collected in the morning, on an empty stomach, after careful hygienic measures in the oral cavity, by spitting in a sterile test tube. Subsequently, the oral fluid in test tubes was centrifuged for 10 minutes at 2000 rpm, and the sediment portion of the fluid was used for biochemical studies.

ADMA concentration was determined using liquid chromatograph LC 5000 (INGOS, Czech Republic), wavelength 340 nm, in isocratic mode. For solid-phase extraction (purification and concentration), Abselut Nexus (Varian) cartridges were used [15].

The activity of lipid peroxidation processes was evaluated by the concentration of TBC-active products. [16]

SOD activity was determined by spectrophotometric method of V.A. Kostiuk, A.N. Potapov and Zh.V. Kovalev, which is based on quercetin oxidation [17].

Catalase activity was determined by spectrophotometric method (according to S. Chevari et al.) [18].

The content of potassium, sodium, calcium, magnesium, total lipids, triglycerides and cholesterol was determined by spectrophotometric methods using a set of reagents of “Filisit – Diagnostika” (Ukraine, Dnipro).

The content of zinc and copper was determined using a set of reagents of DAC-Spectro Med S.R.L (Moldova).

The content of mucin in saliva was determined by spectrophotometric method (according to E.I. Ilyinykh) [19].

The content of lysozyme in saliva was determined using the Micrococus Lysodeicticus culture test, strain N2665 (by the degree of emulsion lightening) [20].

Results and discussion

The differences between the two groups of independent variables were evaluated by Mann Whitney U-criterion.

Table1

Biochemical parameters of saliva of patients with DM-2 (31 people) and DM-2 + hypertension (AH) (9 people) in the morning (8:00). (Me [25- percentile, 75- percentile]).

Indicator	DM-2	DM-2+AH	Reliability
ADMA, μmol/l	0.071 [0.063. 0.081]	0. 141 [0.135. 0.1148]	p = 0.0028
Catalase mmol/sec • g protein	14.45 [13.96. 15.68]	12.22 [11.96. 13.05]	p = 0.0307
SOD, unit/g protein	1.23 [1.11. 1.27]	1.34 [1.17. 1.39]	p = 0.0411
Lysozyme, μmol/l	0.187 [0.179; 0.191]	0.164 [0.159; 0.171]	p = 0.0408
Magnesium, mmol/l	0.345[0.323. 0.362]	0.264 [0.19. 0.0334]	p = 0.0377
Sodium, mmol/l	7.851 [7.562; 8.056]	7.948 [7.669; 8.109]	p = 0.0492
Copper, μmol/l	3.722 [3.453; 3.834]	3.653 [3.411. 3.782]	p = 0.0527
Calcium, mmol/l	1.862 [1.772; 1.902]	1.724 [1.684; 1.786]	p = 0.0477
Protein, g/l	2.611 [2.45; 2.79]	2.309 [2.279; 2.314]	p = 0.0355
Lipids, g/l	0.625 [0.611; 0.636]	0.637 [0.619; 0.644]	p = 0.517
Cholesterol, mmol/l	0.158 [0.146; 0.169]	0.246 [0.238; 0.258]	p =0.0123
Mucin, g/l	2.08 [1.85; 2.12]	1.79 [1.71; 1.92]	p = 0.0477
Urea, mmol/l	1.28 [1.12; 1.34]	1.35 [1.21; 1.38]	p = 0.0614
Uric acid, mmol/l	0.079 [0.071; 0.084]	0.075 [0.067; 0.082]	p = 0.0685
Glucose, mmol/l	0.082 [0.071; 0.087]	0.088 [0.073; 0.092]	p = 0.0712
Lactic acid, mmol/l	0.56 [0.51: 0.63]	0.67 [0.59; 0.74]	p = 0.0469
Cortisol, nmol/l	116.52 [107.18;122.05]	112.65 [105.79;120.03]	P = 0.0521
Adrenalin, nmol/l	1.67 [1.47; 1.79]	2.09 [1.88; 2.21]	p = 0.0417

The sequence of intracellular oxidative-reduction reactions in the body is a finely controlled process. Active forms of oxygen (AFO) are involved in important biological reactions, but their excessive production and accumulation can lead to oxidative stress. Thus, IR is accompanied by a disruption of the AFO metabolism, which leads to the development of oxidative stress. There is evidence that oxidative stress is one of the causes of muscle disorders that contribute to the formation of IR. The importance of AFO and activation of LPO in the pathogenesis of DM2 complications is undeniable. The leading role of hyperglycemia in initiating and potentiating the generation of AFO has been established. There is strong evidence that damage to the β-cells of the Langerhans islets and, as a consequence, insufficiency of insulin secretion, may be due to the activation of oxidative stress mediated by hyperglycemia. The peculiarity of β-cells is low production of antioxidant enzymes, which results in the accumulation of AFO [21].

The data shown in Table 1 indicate that there are significant differences already in the morning between patients with diabetes and diabetes complicated by AH. One the main of these differences is the concentration of ADMA twice as large in the group of patients with DM and AH, which is accompanied by a decrease in the activity of SOD. That is, there are

signs of development of oxidative stress in patients, probably due to the reduction of antioxidant defense.

Table 2.

Biochemical parameters of saliva of patients with DM-2 (31 people) and DM-2 + hypertension (AH) (9 people) in the evening hours (20:00). (Me [25- percentile, 75-percentile]).

Indicator	DM-2	DM-2+AH	Reliability
ADMA, μmol/l	0.112 [0.105; 0.121]	0.155 [0.141; 0.166]	p = 0.0337
Catalase mmol/sec•g protein	11.27 [10.79; 12.18]	14.62 [13.42; 15.71]	p = 0.0412
SOD, unit/g protein	0.81 [0.74; 0.92]	1.37 [1.21; 1.45]	p = 0.0365
Lysozyme, μmol/l	0.164 [0.155; 0.173]	0.137 [0.129; 0.144]	P = 0.0405
Magnesium, mmol/l	0.31 [0.27; 0.35]	0.19 [0.16; 0.24]	p = 0.0392
Sodium, mmol/l	7.65 [7.01; 8.32]	7.86 [7.43; 8.55]	p = 0.0658
Copper, μmol/l	3.88 [3.52; 4.21]	3.54 [3.21; 3.86]	p = 0.0483
Calcium, mmol/l	1.73 [1.57; 1.86]	1.48 [1.33; 1.60]	p = 0.0489
Protein, g/l	2.69 [2.41; 2.91]	2.18 [2.09; 2.31]	p = 0.0 465
Lipids, g/l	0.73 [0.64; 0.81]	0.69 [0.64; 0.75]	p = 0.579
Cholesterol, mmol/l	0.25 [0.22; 0.28]	0.30 [0.26; 0.34]	p = 0.0605
Mucin, g/l	1.59 [1.48; 1.68]	1.78 [1.61; 1.90]	p = 0.0466
Urea, mmol/l	1.39 [1.26; 1.52]	1.47 [1.32; 1.62]	p = 0.0552
Uric acid, mmol/l	0.085 [0.079; 0.092]	0.105 [0.094; 0.114]	p = 0.0492
Glucose, mmol/l	0.097 [0.089; 0.104]	0.092 [0.083; 0.101]	p = 0.0702
Lactic acid, mmol/l	0.66 [0.62; 0.71]	0.72 [0.65; 0.78]	p = 0.0511
Cortisol, nmol/l	127.69 [116.32; 140.13]	126.45 [117.14; 138.32]	p = 0.0714
Adrenalin, nmol/l	1.25 [1.13; 1.37]	1.79 [1.61; 1.92]	p = 0.0447

It should be noted that disturbances are observed in metabolic rate – the level of cholesterol and lactic acid increased. Attention is drawn to the fact that the level of adrenalin was higher in diabetic patients with AH. Indicators in the saliva in the evening (Table 2) according to the main parameters had the same changes. In both groups of patients, the concentration of ADMA increased even more, especially in DM combined with AH, whereas the level of adrenalin, although decreasing toward the evening, but was certainly higher in the second group.

Thus, if we compare the indicators in the saliva collected in the evening, then we can acknowledge the disruptions, which testify to the presence of oxidative stress and changes in FMC in patients, which increase as compared with the healthy ones in patients with diabetes (Fig. 1), and concomitant AH increases the degree of these disorders (Fig. 4).

Figure 2 shows that in patients with diabetes, there are higher concentration of the salivary cortisol, and the accession of AH is accompanied by an increase in the level of

adrenalin, indicating the possible role of activation of the sympathetic and adrenal system in the development of hypertension (Fig. 3).

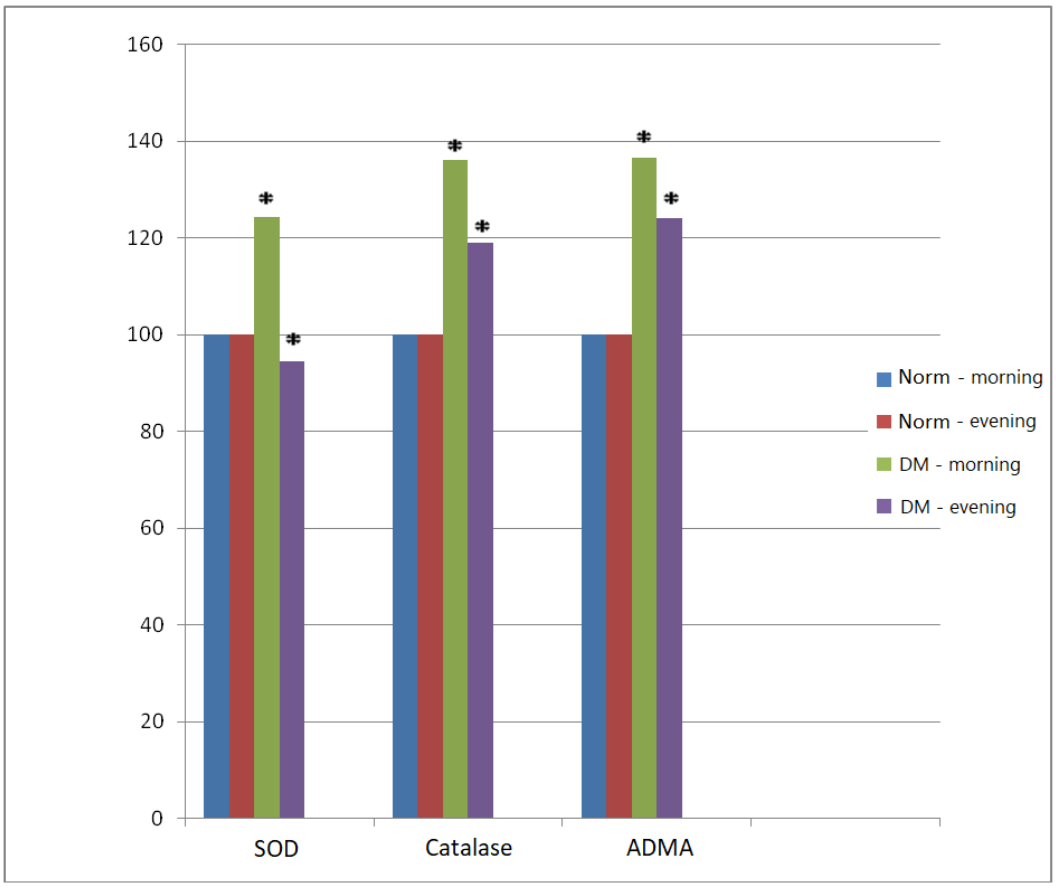

Fig.1

Thus, the study of the circadian rhythm of the parameters in the saliva makes it possible to conclude that in the case of diabetes, and especially with its progress, as evidenced by the accession of AH, complex disruptions of FMC occur. One of its consequences is the development of oxidative stress, which is considered as one of the leading secondary mechanisms in the pathogenesis of diabetes.

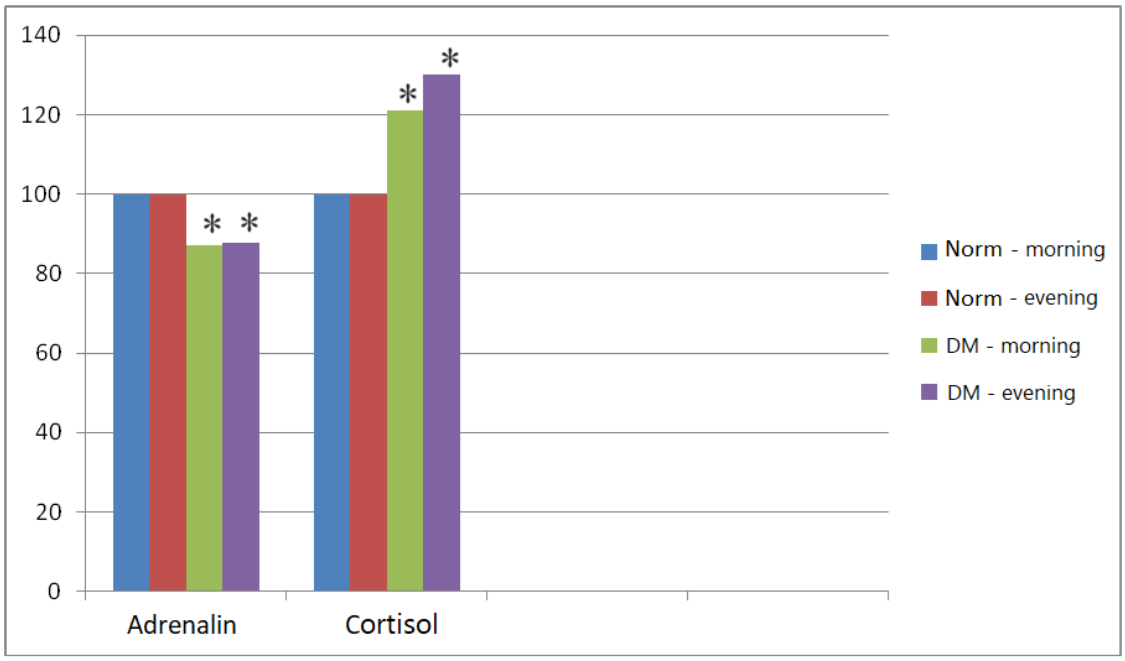
140
120
100
80
60
40
20
0
Adrenalin
Cortisol
Norm - morning
Norm - evening
DM - morning
DM - evening

Fig.2

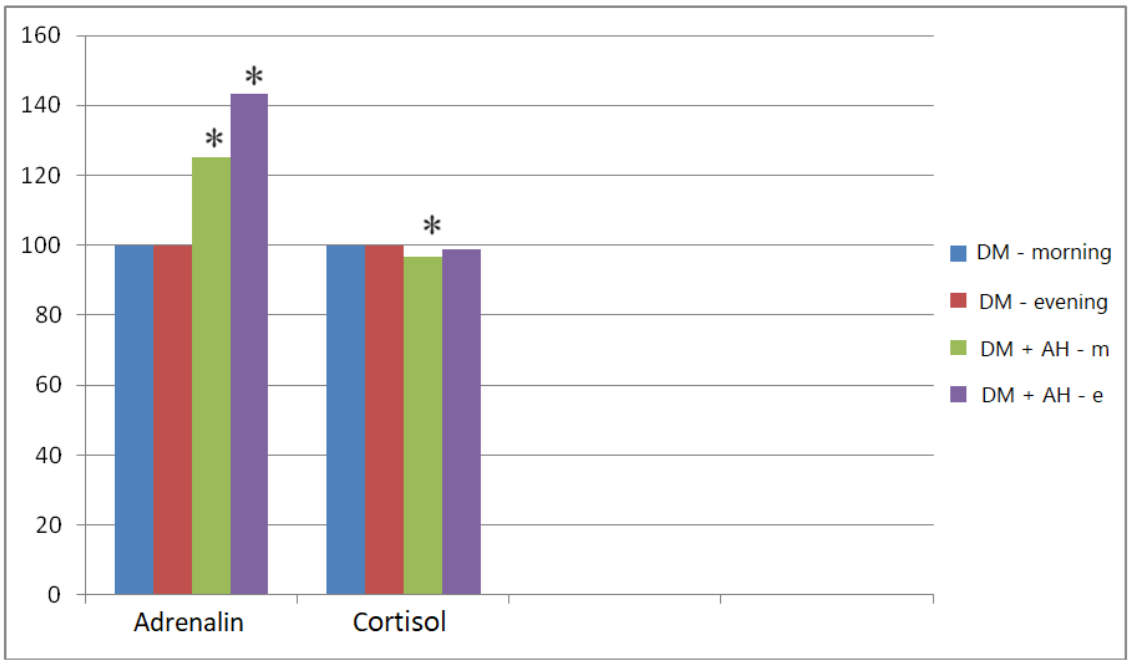
160
140
120
100
80
60
40
20
0
Adrenalin
Cortisol
DM - morning
DM - evening
DM + AH - m
DM + AH - e

Fig. 3

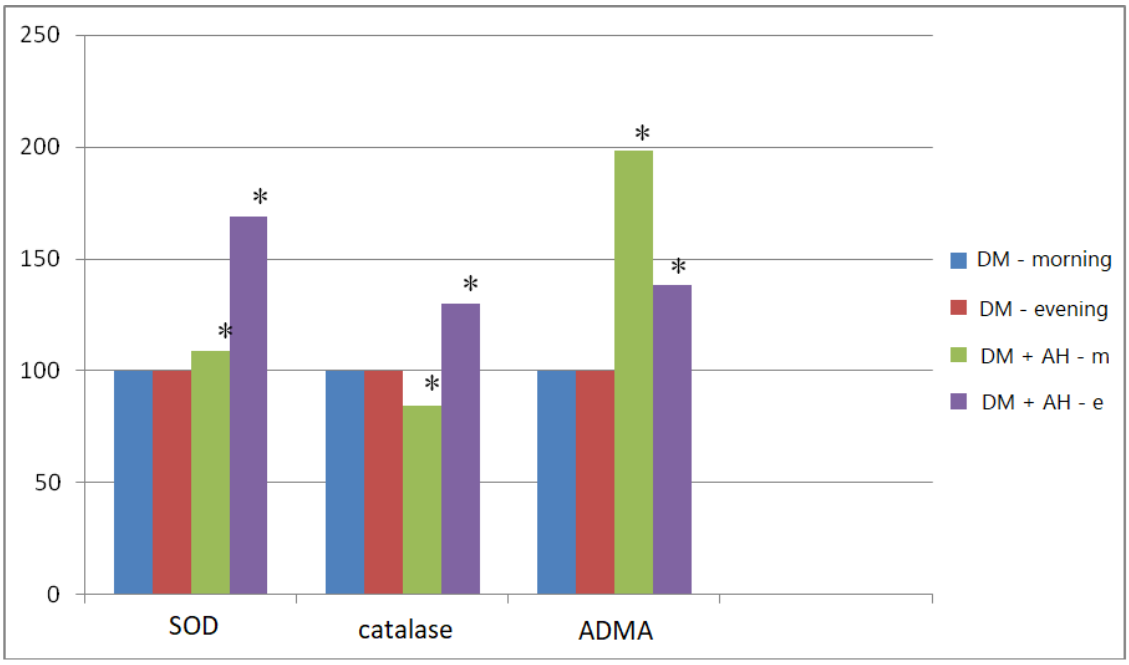

Fig.4

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