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CHANGES IN THE CIRCADIAN RHYTHM OF METABOLIC RATES IN THE SALIVA OF PATIENTS WITH COMPENSATED TYPE 2 DIABETES MELLITUS

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Abstract

In our previous studies, we have shown that salivary parameters differ during the day when one compares the morning and evening samples. That is, the analysis of biochemical components in saliva can be used to monitor the general state of the body's health, which is especially important in metabolic disorders that occur in type 2 diabetes mellitus (DM2). It is possible to assume that the study of daily metabolic changes in patients with type 2 diabetes will help to determine the mechanisms of functional and metabolic continuum (FMC) in this pathology.

Patients with diabetes mellitus present with certain changes in saliva as compared to apparently healthy people. That is, although the daily rhythm of metabolism in saliva remains the same, the metabolic changes occur against this background. They are not significant, but their presence allows us to conclude that even in compensated type 2 DM, changes in metabolism are observed, and this can be explained by the disrupted carbohydrate metabolism with the mobilization of both lipid and protein metabolism. These changes can be observed in the parameters of metabolism in saliva and, especially in the form of a disrupted daily rhythm.

Thus, even in compensated type 2 DM, there are changes in the energy metabolism, induced by hormonal regulation. These changes can be controlled using the daily saliva monitoring.

Key words: diabetes mellitus; daily rhythms; metabolism.

According to the scientific literature, over the past decades there has been deterioration in the health of population, an increase in the incidence of diseases, including an increase in endocrine diseases [1]. Today, diabetes mellitus (DM) presents a serious medical and social problem. Due to the aging of population, increased prevalence of obesity, hypodynamia, consumption of high carbohydrate and fatty foods, the incidence of diabetes in all countries of the world are steadily increasing [2, 3].

DM is among the most common human diseases and is ranked 3rd after atherosclerosis and cancer among the diseases that are the most widespread causes of disability and mortality of patients and is the most acute medical and social problem of the national healthcare system in all countries of the world [1]. It is observed in people of all races on every continent and is manifested in every age group. According to the WHO, out of 6 billion people in the world, 175 million people suffer from diabetes and it is expected that over the next 25 years this number will increase up to 250 million [4]. The WHO states that DM leads to an increase in mortality by 2-3 times and a reduction in life expectancy by 10-30% [1].

Epidemiological studies of diabetes in Ukraine indicate a steady increase in the number of patients. In particular, in 2007, 1.094,124 patients with diabetes were registered, and its prevalence was 2354.7 patients per 100 thousand of population, whereas in 2000, this figure amounted to 1845.8 patients per 100 thousand of population [1].

Until recently, the diagnosis of diabetes, the management of patient's condition and the evaluation of the effectiveness of treatment performed were primarily based on the indicators of blood glucose. The search for simple, accessible, informative and non-invasive methods and biomaterials is a promising direction of modern laboratory diagnostics. Oral fluid is an example of such an accessible biological object of study. [5, 6, 7].

The significance of saliva is much wider than providing the initial stages of digestion. The production and secretion of biologically active substances and hormones by salivary glands has a regulatory effect on most systems of the body. [1].

Previously, we have shown that salivary parameters differ during the day when one compares the morning and evening samples [8]. That is, the analysis of biochemical

components in saliva can be used to monitor the general state of the body's health [9, 10, 11, 12, 13, 14], which is especially important in metabolic disorders that occur in type 2 diabetes. It is possible to assume that the study of daily metabolic changes in patients with type 2 diabetes will help to identify the mechanisms of functional and metabolic continuum (FMC) [8] in this pathology.

The aim of the research

The aim of this research was to study the daily rhythm of functional and metabolic continuum (FMC) in people with type 2 diabetes (duration of the disease from 3 to 5 years, treatment: with metformin) according to the findings of saliva examination.

Research methods

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].

The differences between the two groups of independent indicators were assessed by Student's test.

Results and discussion

Table 1

Metabolism parameters in saliva of patients with diabetes mellitus, duration of the disease from 3 to 5 years, treatment: with metformin, 20 women and 11 men (aged 35-45) in the morning and evening time

Parameter / Time of day	Protein g / l	Lipids g / l	Cholesterol mmol / l	Glucose mmol / l	Urea mmol / l	Uric acid mmol / l	Lactate mmol / l
8-00 n = 21	2.59 ± 0.16	0.62 ± 0.04	0.16 ± 0.01	0.079 ± 0.005	1.25 ± 0.07	0.077 ± 0.006	0.51 ± 0.04
21-00 n= 21	2.66 ± 0.23 P> 0.05	0.71 ± 0.06 P> 0.05	0.24 ± 0.02 P<0.02	0.096 ± 0.006 p<0.05	1.39 ± 0.13 P>0.05	0.084 ± 0.005 P>0.05	0.65 ± 0.04 p< 0.05

Note: p – reliable differences in the value of the parameter in the morning and evening time.

Table 2

Activity of antioxidant system, the content of lysozyme, mucin and some hormones in the saliva of patients with diabetes mellitus, duration of the disease from 3 to 5 years, treatment: with metformin, 20 women and 11 men (aged 35-45) in the morning and evening time

Parameter / Time of day	Catalase Mmol	SOD units/g of protein	Lysozyme µmol / l	Mucin g/ l	Cortisol nmol / l	Adrenalin nmol / l
8-00 n= 21	14.21 ± 1.23	1.18± 0.07	0.189±0.09	1.92 ± 0.07	114.36 ± 7.28	1.64 ± 0.12
21-00 n = 21	11.38 ± 1.02 p<0.05	0.84 ± 0.07 p<0.05	0.166 ± 0.007 P<0.05	1.58 ± 0.09 P<0.02	128.42 ± 5.33 p<0.05	1.28 ± 0.06 P<0.05

Note: p – reliable differences in the value of the parameter in the morning and evening time.

Table 3

The content of ion composition and ADMA in the saliva of patients with type 2 DM, the duration of the disease from 3 to 5 years, treatment: with metformin, 20 women and 11 men (aged 35-45) in the morning and evening time

Parameter / Time of day	Magnesium mmol / l	Sodium mmol / l	Copper μmol / l	Calcium mmol / l	ADMA μmol / l
8-00 n= 21	0.32 ± 0.01	7.94 ± 0.62	3.77 ± 0.29	1.82 ± 0.16	0.071±0.005
20-00 n= 21	0.29 ± 0.02 P>0.05	7.69± 0.68 P>0.05	3.82 ± 0.31 P>0.05	1.71 ± 0.15 P>0.05	0.108±0.009 P<0.02

Note: p – reliable differences in the value of the parameter in the morning and evening time.

Data on the metabolism rates given in Table 1 indicate that in patients with diabetes, significant daily changes are not observed except for the concentration of glucose and lactate, which increase significantly, although in small quantities. The concentration of magnesium, sodium and calcium also do not change (Table 2).

The only increase in the concentration of ADMA by around a half was observed in the evening, and the increase was statistically reliable. Taking into account that the increase of ADMA may be due to the reduction of antioxidant defense, we determined the activity of the main enzymes of catalase and SOD, and it was shown that they were reliably statistically reduced (Table 3).

Taking into account that in type 2 DM the regulation mechanisms of metabolism are disrupted, we determined the concentration of cortisol in the saliva, which significantly increased during the evening, whereas the value of adrenaline, on the contrary, decreased (Table 3).

Thus, in the saliva of patients with type 2 diabetes, certain changes are found as compared with apparently healthy people. Thus, when compared with previously obtained data, the levels of glucose, uric acid, lactate, as well as lipids and cholesterol were elevated [8]. That is, although the daily rhythm of metabolism in saliva remains the same, the metabolic changes occur against this background. They are not significant, but their presence allows us to conclude that even in compensated type 2 DM, changes in metabolism are observed, and this can be explained by the disrupted carbohydrate metabolism with the mobilization of both lipid and protein metabolism. These changes can be observed in the parameters of metabolism in saliva and, especially in the form of a disrupted daily rhythm. As

a result, free radical oxidation is activated, judging by the increase in the concentration of ADMA, which can also be linked to a decrease in the antioxidant defense. In the occurrence of metabolic changes, the leading role belongs to the activation of the pituitary-adrenal system.

It should be emphasized that metabolic changes are observed on the part of energy metabolism parameters, indicating shifts in functional and metabolic continuum (FMC), whereas the regulation of ionic homeostasis is not disrupted (Table 2).

Thus, even in compensated type 2 DM, there are changes in the energy metabolism, induced by hormonal regulation. These changes can be controlled by using the daily saliva monitoring.

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