

Lipid peroxidation and antioxidant system parameters in diabetes mellitus type 2

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Abstract

Experimental and clinical observations proved the participation of reactive oxygen species (ROS) and initiated their lipid peroxidation (LPO) in the pathogenesis of many diseases, including diabetes mellitus. The studies were conducted with the blood of 57 patients with type 2 diabetes (male 21, female 36). All patients were divided into 3 groups based on the duration of the disease, as well as glucose levels: 1 year – compensation stage (n = 27); from 6 to 10 years – the stage subcompensation (n = 12); and more than 10 years – the stage of decompensation (n = 18). The amount of glucose was determined by the glucose oxidase method using ready commercial reagent. As a biochemical marker also was determined level of HbA1c. As a result conducted experiments was determined, that in all 3 stage of disease there is an increased level of LPO, also concentration of MDA, DC. LPO processes were quantitatively investigated using the spectrophotometric method, we determined serum levels of conjugated dyne – primary products of lipid peroxidation and malondialdehyde (MDA) – one of the secondary products. Ferritin levels were determined using a commercial reagent for immunosorbent assay of human ferritin. There is noticeable changes in dates of AOS-decreases the level of reduced glutation, catalase, in contrary increases the level of ceruloplamin. In maximum grade there is increase in level of ferritin – 2.4 times. These dates prove that depending level of glucose in blood increase LPO and decreases AOS of organism.

Key words: diabetes mellitus, ceruloplasmin, ferritin.

Перекисное окисление липидов и показатели антиоксидантной системы при сахарном диабете типа 2

Экспериментальные и клинические наблюдения доказали участие активных форм кислорода и инициируемых ими перекисного окисления липидов в патогенезе многих болезней, в том числе сахарного диабета. Исследования были проведены с кровью 57 пациентов (21 мужчин и 36 женщин) с сахарным диабетом типа 2. Все больные, в зависимости от уровня гликемии и продолжительности заболевания, были разделены на 3 группы: до 1 года – стадия компенсации, (21 чел.), от 6-10 лет – стадия субкомпенсации (12 чел.) и более чем 10 лет – стадия декомпенсации (18 чел.). Количество глюкозы было определено глюкозоксиантным методом с использованием готового коммерческого набора реагента. В качестве биохимического маркера также был определен уровень гликозилированного гемоглобина (HbA1c). Процессы перекисного окисления липидов (ПОЛ) были количественно исследованы с применением спектрофотометрических методов, были определены уровень диеновых конъюгатов – первичных продуктов перекисного окисления и малонового диальдегида (МДА) – одного из вторичных продуктов ПОЛ в плазме крови. Уровень ферритина был установлен с использованием коммерческого набора для иммуноферментного анализа. В результате проведенных исследований выявлено, что на всех 3-х стадиях заболевания происходит нарастание уровня ПОЛ, возрастает концентрация МДА и ДК. Заметное изменение происходит по показателям АОС – снижается уровень восстановленного глутатиона, каталазы и наоборот, увеличивается уровень церулоплазмина. В наибольшей степени увеличивается уровень ферритина в 2,4 раза. Эти данные доказывают, что в зависимости от уровня глюкозы в крови усиливается ПОЛ и снижается АОС организма.

Ключевые слова: сахарный диабет, церулоплазмин, ферритин.

Introduction

According to modern ideas, free radical reactions attribute to fundamental processes, ensuring normal functioning of an organism and are involved in the development of most forms of pathology. Numerous experimental and clinical observations proved the participation of reactive oxygen species (ROS) and initiated their lipid peroxidation (LPO) in the pathogenesis of many diseases, including diabetes mellitus [1, 2]. The most important link of pathogenesis of diabetes mellitus (DM) is a destabilization of membrane structures, characterized by quantitative and qualitative changes in the lipid bilayer plasma membrane of cells. Various etiological factors, such as inflammation, tissue ischemia leads to changes in the activity of membrane enzymes, which can lead to shifts in the lipid structure of cell membranes and activation of lipid peroxidation, which in turn forms a symptom of renal impairment, intoxication, and immune deficiency [3]. Disruption of the normal functioning of the immune system contributes to the generalization of inflammatory processes, development of complications, lack or loss of clinical effect of basic therapy, and an increase in mortality.

In the pathogenesis of diabetes, imbalance between lipid peroxidation and antioxidant system (AOS) plays a significant role. Reasons for the increase of LPO in the organs and tissues can be attributed to either increased generation of reactive oxygen metabolites by neutrophils or the lack of effectiveness of antioxidants [4]. Diabetes mellitus remains one of the predominate conditions of clinical medicine and public health in connection with an increasing number of patients in all countries, including Azerbaijan. According to WHO, there are more than 175 million patients with diabetes. Expert assessment of the prevalence of the disease suggests that by 2030 there will be more than 230 million patients diagnosed with diabetes, and by 2025 300 million diabetic patients will be diagnosed, of whom 80-90% will be patients with diabetes mellitus type 2 [5, 10].

The purpose of this study was to investigate the state of lipid peroxidation and antioxidant system in blood plasma of patients with type 2 diabetes mellitus.

Material and methods

The studies were conducted with the blood of 57 patients with type 2 diabetes (male 21, female 36). The bulk of patients

were treated in the self-supporting Endocrinology Dispensary, Baku. All patients were divided into 3 groups based on the duration of the disease, as well as glucose levels: 1 year – compensation stage (n = 27); from 6 to 10 years – the stage subcompensation (n = 12); and more than 10 years – the stage of decompensation (n = 18). The amount of glucose was determined by the glucose oxidase method, ready to jet-set [2]. Concentration of HbA1 was determined by special methods [3]. The duration of disease was determined by the amount of glucose and HbA1 [6]. LPO processes were quantitatively investigated by spectrophotometric method; we determined serum levels of conjugated dienes – primary products of lipid peroxidation and malondialdehyde (MDA) – one of the secondary products. The principle method for the determination of malondialdehyde was based on the formation of colored trimetin complex with thiobarbituric acid at high temperatures [7]. The optical density of the colored complex after its extraction with n-butanol was carried out at a length of 532 nm in comparison with control samples. Molar extinction of MDA 1.56 M-1 cm-1. The concentration of malondialdehyde was expressed in nmol/ml serum. The state of AOS was determined by the level in the serum of patients with superoxide dismutase (SOD) and GP [9]. In addition, the measured integral parameters balance of LPO and AOS, which is used to calculate the mathematical equation where one side contains the product of the relative values of lipid peroxidation, and the other indicators of AOS. The level of copper-containing enzyme ceruloplasmin (Cp) was determined by Revin [8]. Ferritin levels were determined using a commercial reagent for immunosorbent assay of human ferritin.

Results and discussion

The resulting biochemical data from the conducted experiments are presented in table 1.

With prolonged and persistent violation of carbohydrate metabolism, decompensation of diabetes mellitus and the lack of adequate correction of the disease increased the levels of HbA1. In parallel, non-enzymatic glycosylation of hemoglobin and other proteins is an organism that can cause

Table 1

Blood glucose levels and glycated hemoglobin in patients with type 2 diabetes

Group of diabetic patients	Number of patients	Average daily blood glucose, mmol/l	HbA1C%
In the state compensation	27	5.74 ± 0.14	6.5 ± 0.55
Subcompensation	12	8.1 ± 0.18 *	7.7 ± 3.31
Decompensation	18	8.74 ± 0.58	10.5 ± 0.82**
Control	20	3.8 ± 0.22	5.4 ± 0.91

*p < 0,05,**p < 0,005

such changes characteristic of the progression of diabetes as a thickening of the membrane, metabolic disorders. Indicators of LPO and AOS are shown in table 2.

DM patients exhibited regular increases in the content of MDA and DC in the blood serum with maximal values in patients with diabetes at the stage of decompensation. These changes have taken place against the background of the main biochemical parameters – increase in the level of glucose in the blood serum. The level of reduced glutathione (GSH), on the contrary, decreases depending on the severity of the disease. Thus, the MDA level in diabetic patients in group I increased by 1.6 times, in the group II 2.4 times and in group III 3.1 times. Almost the same dynamics of change were observed for diene conjugates (DC). In patients with conservative groups an increase of 1.8 times was observed; whereas in the terminal group an increase of 2.4 times was observed.

The level of CP (ceruloplasmin) in the blood plasma of patients with CD was reduced in comparison with indicators of healthy patients, after applying the appropriate treatment, a noticeable improvement was observed. Apparently, this is due to a well-compensated response of the body upon the activation of LPO processes at this stage of the disease. In stage III of the disease there was a significant reduction level of CP.

In this study, the determination of the activity of glutathi-

Table 2

LPO and AOS in patients with type 2 diabetes compared with the control

Dates	Control	In the state compensation	Subcompensation	Decompensation
MDA, nmol/l	3.7 ± 0.1	6.01 ± 1.22*	8.69 ± 1.87*	12.48 ± 1.54*
DK, E223/ml	0.46 ± 0.02	0.48 ± 0.02*	0.82 ± 0.02*	1.1 ± 0.02*
GSH mkmol/l	1.89 ± 0.04	1.53 ± 0.18*	1.44 ± 0.21*	1.29 ± 0.19
SOD, IU/mq	806 ± 23.14	986 ± 193*	998 ± 122*	1195 ± 166*
CAT	72.98 ± 2.32	51.64 ± 9.81*	49.16 ± 8.41*	60.91 ± 13.20*
GPO, U/qHb	46.8 ± 1.04	59.23 ± 11.2*	62.69 ± 8.63*	65.78 ± 9.30*
Ceruloplazmin, mkq%	17.85 ± 0.61	35.57 ± 9.50*	32.25 ± 10.43*	31.54 ± 9.57*
Ferritin, nq/ml	139.93 ± 3.58	314.98 ± 97.54*	234.27 ± 87.38*	329.72 ± 82.87*

*p < 0,05

one peroxidase in the blood serum of patients with diabetes was carried out using hydrogen peroxide and 5.5'-dithiobis (2-nitrobenzoic acid [5]. Selenium glutathione peroxidases (GPO) are a group of enzymes catalyzing the recovery of hydrogen peroxide (H₂O₂) and organic peroxides, used as an electron donor for the reduced form of glutathione (GSH). Selenocysteine is present in the enzymes of this group, which makes them sensitive to the content of selenium in tissues. It is known that selenium is an essential element for the biosynthesis of seleno-dependent GPO. A deficiency of selenium causes a decrease in the level determined by the glutathione peroxidase activity in many organs and tissues. Selenium of GPO interacts effectively with a wide range of compounds containing peroxide groups. To determine the activity of these enzymes, often several organic peroxides are used. At the same time, selenium GPO is not the only group of enzymes that can restore the peroxides formed in vivo. The recovery of peroxide groups of biomolecules and other functional groups related to SH groups of glutathione (GSH) and peroxiredoxins, which in the aggregate may be designated as non-seleno-containing enzymes. In some tissues, the glutathione peroxidase activity is comparable to or even exceeds the activity of enzymes with selenium forming the same reactions.

Ferritin is the major protein of the human body stocking with a molecular weight 450000. It is characterized by a strong tertiary structure close to spherical, aimed at preserving large amounts of iron in a soluble, easily metabolized form. All ferritin interact with iron (II) in the reaction catalyzed in

a ferroxidase center, inducing its oxidation and deposition in the cavity in a mineral form. Thus, depending on the glycemia level in patients with type 2 diabetes, observations were made of increased LPO processes and reducing components AOC system, which determines the complications of diabetes.

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Aspectul terapeutic al Ronocitului (CDP-cholina) la copiii cu cefalee migrenoasă

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Therapeutic view of Ronocit (CDP-choline) in children with migraine headaches

We performed a study of the frequency and duration of migraine headaches and specific changes in EEG and dynamics of laboratory changes in serum of cardiolipin, phosphatidylcholine, sphingomyelin and α -fetoprotein with the immunofluorescence screening analysis method (ELISA) before and after treatment. Ronocit was administered parenterally and orally for a period of 40 days at a dose of 1000 mg in two divided doses. While reducing the frequency and duration of headaches in children, an increased lipid metabolism was observed, especially of phospholipids. A parenteral treatment period of 10 days in patients with migraine headaches were compared with the results from the treatment of children with Cerebral Palsy. By analyzing side effects, of Ronocit in children with migraine headaches and cerebral palsy, we can conclude that no side effects were noted. There was a clinical and neurophysiological (EEG) improvement in the frequency and duration of migraine access, while increasing antioxidant defense system. This aspect can be used for prophylactic migraine access in children.

Key words: Ronocit, migraine, headache, access, immunofluorescence screening.