UDC 616.379-008.64-002:577.2 doi: https://doi.org/10.15407/ubj95.02.048

OXIDATIVE STRESS IN TYPE 2 DIABETIC PATIENTS: INVOLVEMENT OF HIF-1 ALPHA AND mTOR GENES EXPRESSION

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Received: 22 March 2023; **Revised:** 25 May 2023; **Accepted:** 05 June 2023

Biochemical and genetic mechanisms of oxidative stress (OS) developing in the blood of patients with type 2 Diabetes mellitus (T2DM) were studied. Twenty patients with T2DM and 10 healthy persons participated in this study. Lipid peroxidation, the content of protein carbonyls and H_2O_2 production were measured in blood plasma and erythrocytes as OS biomarkers. Activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GP_x) as well as reduced glutathione (GSH) level in plasma and erythrocytes were estimated. The gene expression of key regulators of oxygen and metabolic homeostasis (HIF-1α and mTOR) in leukocytes were studied. It was found a significant rise in TBARS and protein carbonyls content in plasma as well as H_2O_2 production in erythrocytes from patients with T2DM compared to control. The diabetic patients also demonstrated an increase in the SOD and catalase activity in plasma and significantly lower GSH content and GP_x activity in erythrocytes compared to the healthy participants. The established marked inhibition of mTOR gene expression and the tendency to an increase in HIF-1α gene expression in leukocytes of patients with T2DM may serve as a protective mechanism which counteracts OS developing and oxidative cell damage.

K e y w o r d s: oxidative stress, HIF-1α, mTOR, type 2 Diabetes mellitus.

Oxidative stress (OS) is caused by an imbalance in reactive oxygen species (ROS) generation and ROS-degrading pathways resulting in oxidative damage of cellular proteins, lipids, and DNA. OS is one of the major factors in the pathogenesis of type 2 Diabetes mellitus (T2DM) and its complications [1, 2, 3]. In the diabetic milieu, hyperglycemia enhances the production of ROS in various tissues [2]. Intracellular metabolic mechanisms, such as hyperglycemia-induced enhancement of the polyol pathway, protein kinase C and hexosamine pathway activation, excessive production of advanced glycation end-products, and high levels of free fatty acids have been implicated in the overproduction of ROS in diabetic tissues [4, 5]. Accumulating evidence suggests that tissue hypoxia and impaired adaptive responses to hypoxia could both contribute to the increased ROS production in multiple tissues of patients with T2DM [6]. Whereas evidence for increased ROS production in T2DM is reasonably strong, the effect of this disease on antioxidant defenses is controversial. Thus, the activity levels of copper/zinc- superoxide dismutase, glutathione peroxidase, or catalase in blood of patients with T2DM were either increased [7, 8] or decreased [9, 10]. Accumulating evidence has demonstrated the tight relationship between alterations in the activity of antioxidant enzymes in blood of patients with T2DM and duration and severity of the disease [11]. It is widely known that glutathione system is one of the active components of antioxidant defense which acts to maintain the intracellular thiol redox balance and thus protects the cell against oxidative injury. The studies of glutathione system in blood of patients with T2DM have also shown the conflicting results [12, 13].

As mentioned earlier, the impairment of adaptive responses to hypoxia in diabetes could be, in

part, due to the dysregulation of hypoxia-inducible factors (HIFs) signaling [14]. HIFs are heterodimeric proteins, consisting of oxygen-sensitive α-subunits (HIF-1, -2, and -3) and an oxygen-independent constitutively expressed HIF-1β subunit. Several studies have shown that HIF-1α expression was modified in hypoxic tissues of individuals with T2DM [15, 16]. Recently, the therapeutic potential of pharmacological targeting HIF signaling was established in diabetes [6] but further research is required to clarify the impact of the HIFs system changes on OS developing in diabetic tissues.

Similar to the importance of controlling the degree of activity for HIFs, controlled activity of mTOR (the mammalian target of rapamycin) - the master regulator of metabolic state may also be very important in a number of metabolic pathological conditions, including obesity, cancer, neurological diseases, and T2DM [5, 17, 18]. mTOR is the conserved serine/threonine protein kinase which exists in two multi-complexes mTORC1 and mTORC2 with different protein components and downstream substrates. Both two complexes directly influence either the development or the protection of OS in different tissues of patients with Alzheimer's disease, metabolic syndrome, and T2DM, acting through different signaling pathways (HIF-1α, EPO, AMPK, SIRT1, etc) [5, 18, 19]. At the same time, further work is necessary to examine whether mTOR gene expression is involved in characterization of blood oxidative stress in type 2 Diabetes mellitus patients.

To summarize, we must underline that the data concerning both the components of antiradical defense and glutathione antioxidant system in blood of patients with T2DM are still somewhat controversial and require further study. The issue of changed HIF-1 α and mTOR genes expression as a means to control OS developing also requires clarification.

This study was designed to investigate the pro- and antioxidant balance changes and OS as well as the gene expression of key regulators of oxygen and metabolic homeostasis (HIF-1α and mTOR) in blood of patients with T2DM.

Materials and Methods

*Design of th*e Study and Description of the Study Contingent. The study examined 20 patients with T2DM and 10 healthy subjects (control).

All experimental procedures were performed in accordance with the policy statement of the SI "The Scientific and Practical Medical Center of Pediatric Cardiology and Cardiac Surgery of the Ministry of Health of Ukraine. Clinic for adults" and approved by the bioethics committee of this Institution (Protocol N 0025/615-21.05.2020). Written informed consent was obtained from each of the participating subjects.

All patients were treated with oral antihyperglycemic medications such as metformin, sulfonylurea, SGLT-2 inhibitors and six patients received basal insulin. All patients received statins and antihypertensives (ACE-inhibitors, angiotensin receptor blockers, diuretics, and calcium antagonists).

Blood samples were collected from the patients and controls at the same time in the morning after overnight fasting. The samples were obtained from the antecubital vein into ethylenediaminetetraacetic acid (EDTA) tubes, and immediately centrifuged at 3000 g at 4°C for 10 min. After centrifugation, plasma and erythrocytes were separated. Plasma was frozen and stored at –80° until analysis. The buffy coat on the erythrocyte sediment was carefully separated. The erythrocytes were subsequently washed twice with two volumes of 0.9% sodium chloride solution to remove the plasma remnants. Following this, the erythrocytes were hemolyzed with two-fold volumes of ice-cold distilled water. After centrifugation (5000 g, 10 min, 4°C), the supernatant was subdivided and transferred into polyethylene tubes. The hemoglobin (Hb) content was also measured (Hemoglobin Assay kit MAK115, Sigma-Aldrich, St. Louis, MO, USA). All assays were performed in duplicate and on the first thaw.

Glycated hemoglobin (HbA1c) and low-density lipoprotein (LDL) levels were estimated using the automatic analyzer ARCHITECT C-800 Abbot Diagnostic (USA) and the test kits Abbot (USA).

Chemicals. Analytical grade chemicals were purchased from Sigma – Aldrich (St. Louis, MO, USA) for use in the biochemical analyses.

Oxidative stress biomarkers assay. Lipid peroxidation in plasma was measured from the formation of thiobarbituric acid – reactive substances (TBARS) using the method of Buege and Aust [20]. TBARS were isolated by boiling plasma samples for 15 min at 100°C with the thiobarbituric acid reagent (0.5% 2-thiobarbituric acid/10.0% trichloroacetic acid/0.63 mM hydrochloric acid) and measuring the absorbance at 532 nm. The results are expressed as μM/ml using $\varepsilon = 1.56 \times 10^5$ $mM^{-1}cm^{-1}$.

Protein carbonyls (the products of oxidative protein modification, OPM) in plasma were detected

by their reaction with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of protein hydrazones [21].The absorbance of the samples was measured at 370 nm. Carbonyl contents were calculated using the molar extinction coefficient of DNPH, $\varepsilon = 22\times10^3\ M^{-1}cm^{-1}$.

H_2O_2 concentration in erythrocytes was measured using the FOX method [22], which is based on the peroxide-mediated oxidation of Fe^{2+}, followed by a reaction of Fe^{3+} with xylenol orange (*o*-cresolsulfonephthalein 3′,3′bis[methylimino] diacetic acid, sodium salt). This method is extremely sensitive and used to measure low levels of water-soluble hydroperoxide present in the aqueous phase. The absorbance of the Fe^{3+}-xylenol orange complex was detected at 560 nm. The data were normalized and expressed as μM H_2O_2 per mg of Hb [22].

Enzymatic assays. Total superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured in plasma by Misra and Fridovich method [23], which is based on the inhibition of autooxidation of adrenaline to adrenochrome by SOD contained in the examined samples. The results were expressed as specific activity of the enzyme in units per ml of plasma. One unit of SOD activity is defined as the amount of protein causing 50% inhibition of the conversion rate of adrenaline to adrenochrome under specified conditions.

Catalase (EC 1.11.1.6) activity was measured in plasma by the decomposition of hydrogen peroxide, determined by a decrease in the absorbance at 240 nm [24].

The activity of selenium-dependent glutathione peroxidase (GP_x) (EC 1.11.1.9) was determined in erythrocytes according to the method [25]. Briefly, the reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 mM GSH, 0.25 mM H_2O_2, 226 U/ml glutathione reductase; rates of NADPH oxidation followed at 340 nm.

Measurement of the reduced glutathione level. The reduced glutathione (GSH) was determined as described [26]. The erythrocytes sample was mixed with sulfosalicylic acid (4%) and incubated at 4°C for 30 min. Thereafter, the mixture was centrifuged at 1200 g for 15 min at 4°C, and 0.1 ml of this supernatant was added to phosphate buffer (0.1 M, pH 7.4) containing DTNB in abs. ethanol. The yellow color that developed was read immediately at 412 nm. The GSH content was calculated as μM GSH per mg of Hb ($\varepsilon = 13.6\times10^3\ M^{-1}cm^{-1}$).

Determination of gene expression. mRNA expression of HIF-1α and mTOR was determined in circulated blood leukocytes. Leukocytes were obtained by centrifugation of blood samples at 1500 g for 15 min. After centrifugation, the supernatant with interphase fraction was collected and transferred into new tubes. After a secondary centrifugation (3000 g for 3 min), the supernatant was removed, the precipitate was used for RNA isolation. Total RNA was isolated from the leukocyte fraction using the TRIzol kit (Invitrogen, USA). The method is based on Trizol-reagent containing guanidine isothiocyanate, intended for cell lysis, solubilization of cell debris, and denaturation of cellular ribonucleases and proteins. Total RNA is then extracted into a solution of phenol-chloroform by centrifugation, washed from proteins, and transferred to sterile, DNA and RNA-free microtubes. The resulting RNA can be used directly for reverse transcription. The kit allows for isolating from fresh biological material high molecular weight native RNA of high purity (OD 260/280 nm 2.0). Reverse transcription was performed using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific ™, USA) using a 1.2-1.5 μg total RNA and hexameric primer. The resulting single-stranded DNA was used to quantify gene expression by performing real-time polymerase chain reaction and using unique probes for the HIF-1α and mTOR genes (Hs00153153_m1 and Hs00234508_m1).

Amplification was performed using a thermal cycler "7500 Fast Real-Time PCR System". Kits that were developed based on the human mRNA sequences designed by Applied Biosystems (USA) and used to quantify gene expression. The expression level of HIF-1α and mTOR genes was normalized to the expression of the reference gene β-actin (Hs03928985_g1) using a Tag Man human β-actin control reagent. The relative gene expression level was calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$).

Statistical analysis. The difference between the patients and controls parameters was detected by Student's independent *t* test. Data are expressed as mean ± SD. A *P* value of less than 0.05 was considered significant.

Table. Clinical and anthropometric data of the subjects

Indicators	T2DM, $n = 20$	*Controls, n = 10*
Age, years	61.6 ± 2.5*	50.00 ± 2.73
Sex: Male/Female	8 (40%)/ 12 (60%)	4 (40%)/ 6 (60%)
Weight, kg	79.9 ± 3.8	75.8 ± 2.8
BMI, kg/m^2	30.17 ± 1.23	28.30 ± 2.18
SAP, mmHg	140.20 ± 4.23	135.30 ± 3.25
DAP, mmHg	81.90 ± 1.92	82.70 ± 1.81
HbA1c, %	9.90 ± 0.67*	5.50 ± 0.62
Diabetes duration, years	13.90 ± 1.61	–
LDL, mmol/l	2.40 ± 0.23	2.10 ± 0.18
Smoking Habit, n%	45	50

Note. Data are mean ± SD; *$P < 0.05$ vs healthy controls; LDL – low-density lipoprotein; BMI – body mass index; SAP – systolic arterial pressure; DAP – diastolic arterial pressure; HbA1c – glycated hemoglobin

Results

The clinical and anthropometric characteristics of the subjects studied are displayed in Table.

Both groups displayed the same gender distribution and a similar body mass index (less than 40 kg/m^2). Blood concentration of HbA1c in patients with T2DM increased by 80% compared to control ($P < 0.05$).

We found that the indices of oxidative stress increased significantly in the blood of patients with diabetes compared to the healthy participants. Thus, the levels of TBARS in plasma and H_2O_2 in erythrocytes of patients with diabetes were increased by 54 and 23%, respectively, as compared to control ($P < 0.05$) (Fig. 1, *A*; Fig. 2, *A*).

As shown in Fig. 1, *B*, a significant increase in protein carbonyls value was found in plasma of patients with T2DM (by 35%) compared to controls ($P < 0.05$). A significant increase in the total SOD activity (by 31%) was registered in T2DM patients in comparison to control group ($P < 0.05$) (Fig. 1, *C*). A similar result was registered in the catalase activity, revealing its increase by 83% as compared to controls ($P < 0.05$) (Fig. 1, *D*). We found that patients with diabetes demonstrated significantly lower values of GSH content (by 32%, $P < 0.05$) and activity of glutathione peroxidase (by 16%, $P > 0.05$) than control subjects (Fig. 2, *B*, *C*). However, the increase in the activity of antioxidant enzymes such as SOD and catalase in T2DM is not sufficient to protect cells against oxidative stress, because increased LPO and OMP levels, depleted GSH and decreased GP_x activity indicate that oxidative damage has already occurred.

We have found a trend toward the increase (by 22%) in relative HIF-1α expression in leukocytes of patients with T2DM in comparison to controls ($0.1 > P > 0.05$) (Fig. 3, *A*). Patients with T2DM also showed a significant reduction (by 52%) of relative mTOR gene expression level in leukocytes in comparison to controls ($P < 0.05$) (Fig. 3, *B*).

Discussion

Patients with T2DM showed a significantly increased plasma concentration of protein carbonyls - the products of OPM. OPM is known to be a very sensitive and reliable biomarker of cumulative oxidative stress [27]. OPM is realized via various mechanisms: direct oxidation of amino acid side chains, modification of side chains with lipid peroxidation products or with products of protein glycation and glycoxidation [28]. Carbonyl intermediates (glyoxal, methylglyoxal, 3-deoxyglucozone) are responsible for protein glycoxidation, forming end-products of non-enzymatic glycation which, in turn, may lead to enhancement of cellular prooxidant processes and superoxide anion radicals generation [29]. Oxidative-modified proteins may serve as an additional source of ROS and exhaust the reserves of endogenous antioxidants (ascorbic acid, glutathione) [27, 29]. The early clinical studies have shown that significant elevation in protein carbonyls content was registered in blood of patients with the most serious complication of T2DM [30, 31]. In our study, patients with T2DM also exhibited a significant increase in the content of the secondary products of lipid peroxidation in plasma as well as in H_2O_2 production in erythrocytes as compared to control. Our findings correlate well with the earlier studies showing the significant increase in serum malondialdehyde content in patients with T2DM [32-35]. Al-Nimer (2012) et al. have found the high levels of peroxinitrite in the blood and urine of patients with T2DM that could be considered as a confirmation of nitrosative and oxidative stress developing in pathological conditions [36]. It is well-known that oxidative damage includes alteration, predominantly, in the proteins, lipids, and DNA

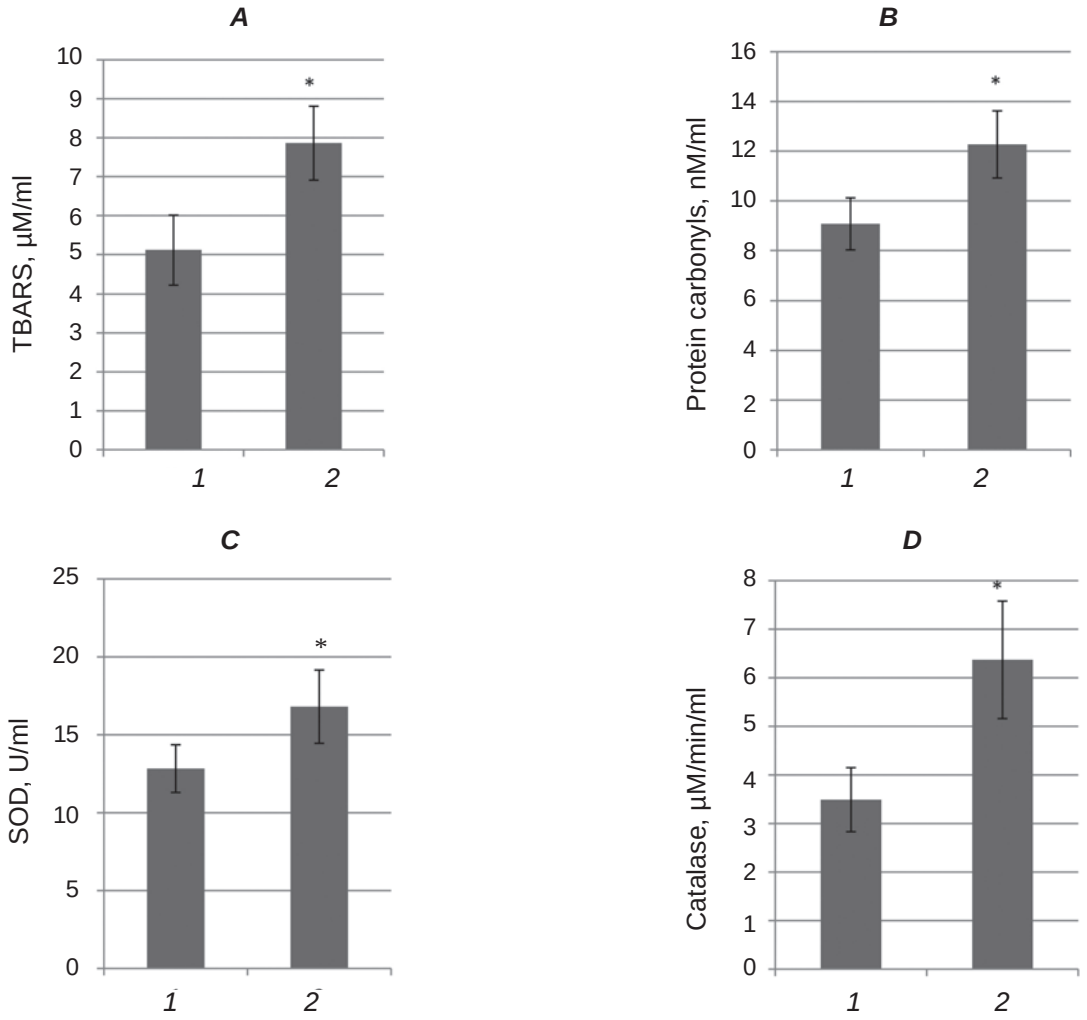

*Fig. 1. Parameters of oxidative and anti-oxidative status in plasma of different study groups. 1 – healthy control (n = 10); 2 – patients with T2DM (n = 20). *P < 0.05 vs healthy controls*

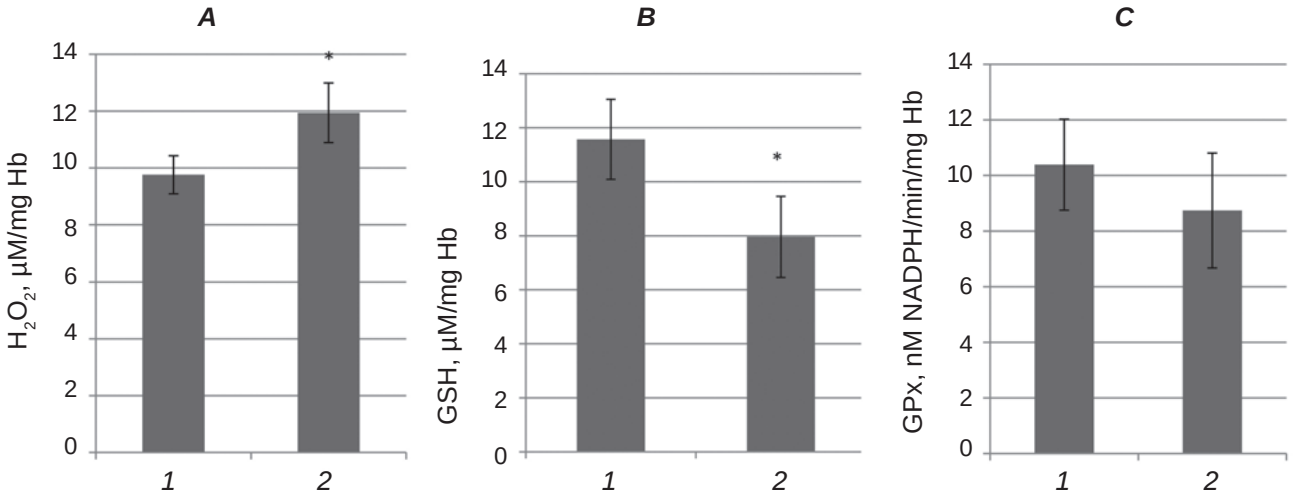

*Fig. 2. Levels of oxidative stress markers in erythrocytes of different study groups. 1 – healthy control (n = 10); 2 – patients with T2DM (n = 20). *P < 0.05 vs healthy controls*

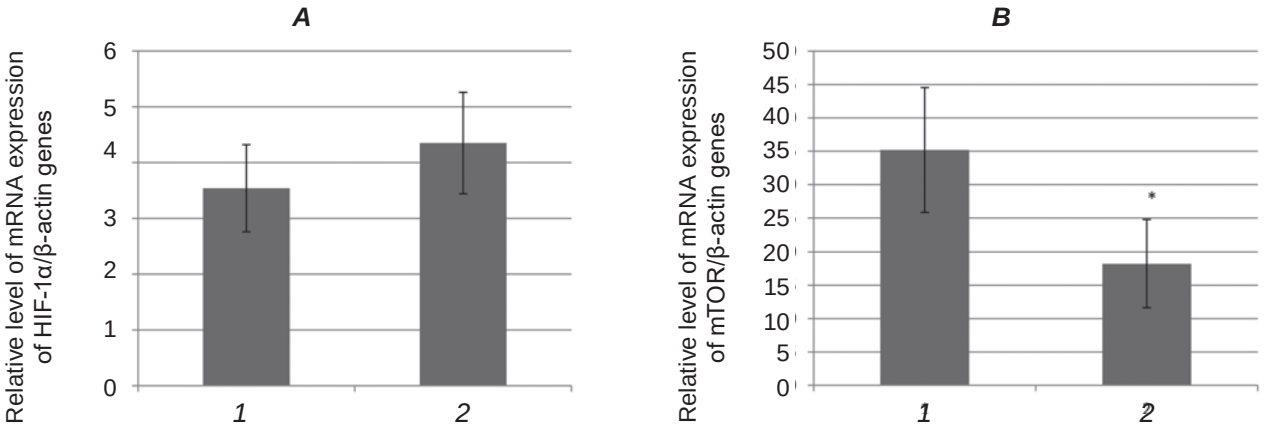

*Fig. 3. Relative HIF-1 (**A**) and mTOR (**B**) gene expression in leukocytes of patients with T2DM. 1 – healthy control (n = 10); 2 – patients with T2DM (n = 20).Values are means ± SD (n = 20).*P < 0.05 vs healthy controls*

structure and function. Oxidative damage to DNA can cause structural modifications of the nucleotide bases or cross-linking that can lead to gene mutation and cell damage. Oxidative damage also can modify amino acid side chains and lead to protein breakdown increasing chemical fragmentation and enhancing susceptibility to proteolytic attack [1]. Lipid peroxidation can occur as a chain reaction that can self-perpetuate, thereby amplifying an initial oxygen radical insult by severalfold [3]. Unstable lipid peroxides derived from polyunsaturated fatty acid breakdown to several compounds (isoprostanes, alkanals, etc) which might have adverse vascular effect in diabetes [37]. We suggest that in patients with T2DM the marked elevation in OS intensity was connected not only with overproduction of ROS but also with the changes in antioxidant systems. Because of the important role of antioxidant enzymes SOD and catalase as the first line of antioxidant cell defense, we investigated their activities in blood of diabetic patients. Significant activation of these enzymes witness for overgeneration of primary ROS-superoxide anion, which serves as a substrate for SOD as well as H_2O_2, which serves as a substrate for catalase. These results correlate well with the analogous investigations of other authors [2, 32, 34]. We suggest that the established rise in SOD and catalase activities can be explained as a compensatory reaction to the increased production of superoxide anion and H_2O_2 in diabetic patients. Additionally, we have found a decrease in GSH level and GP_x activity in erythrocytes of patients with T2DM in comparison with the control values. Similar findings were also reported by Mendez et al. in patients with T2DM [13]. It is known that the level of reduced glutathione in the cell is supported by two ways: the first –by synthesis of GSH *de novo*, and the second – by its reduction from GSSG in glutathione reductase reaction using NADPH. In the diabetic state, hyperglycemia induces enhancement of the polyol pathway of glucose oxidation with the NADPH exhaustion as well as enhancement of glucose autooxidation accompanied by an increase in GSSG and a decrease in GSH content [12]. We can assume that the established decrease in GP_x activity may induce OS developing in erythrocytes due to a decreased removal of H_2O_2. In erythrocytes of patients with T2DM, the exhaustion of glutathione pool may indicate the existence of significant intracellular redox state impairment and imbalance in the pro/antioxidant system with intensification of prooxidant processes.

We have found that patients with T2DM showed a trend toward an increase in HIF-1α gene expression in blood leukocytes. It is known that multiple tissues are hypoxic in diabetes [14, 15]. Hypoxia is well-known to initiate the adaptive gene transcription program via HIFs among which HIF-1α triggers hypoxia-dependent gene expression in regulating many metabolic processes for the improvement of oxygen transport and utilization capacity as well as for glucose homeostasis and insulin resistance [6, 14, 15, 38]. It was also shown that HIF-1α plays a significant role in the prevention of ROS overproduction due to direct and indirect mechanisms: by suppressing mitochondrial biogenesis and activating selective autophagy of mitochondria (mitophagy), by modulating the cytochrome C-oxidase subunits expression as well as by regulating PDK1 induction [16].

It was demonstrated that the structurally different pharmacologic agents that induced HIF-1α and mRNAs regulated by HIF-1α may stimulate aerobic glycolysis, which results in the decreased ROS formation [14]. This was explained by two cooperative effects: an increase in antioxidant pyruvate and prevention of ROS production by reducing the rate of glucose oxidation. So, the established tendency to an increase in HIF-1α gene expression in leukocytes of diabetic patients may represent a mechanism of minimization of oxidative cell damage. On the other hand, our findings have demonstrated a blunted level of HIF-1α gene expression in T2DM which could be due to the dysregulation of HIF-1α signaling [31]. The underlying mechanisms of such dysregulation are mainly connected with hyperglycemia and high levels of fatty acids (palmitate or oleate) which can promote prolyl hydroxylase-mediated HIF-1α degradation [6]. There are recent experimental studies about a role of impaired HIF-1α signaling in diabetic tissues connected with OS development. These studies have shown that mouse embryonic fibroblasts lacking HIF-1α gene undergo cell death as a result of excess ROS production [39, 40]. Inhibited HIF-1α signaling contributes to impaired wound healing in mouse model of diabetes accelerating oxidative stress [41]. Recent clinical investigations have shown that in T2DM the gene expression of HIF-1α correlates with the degree of metabolic control [15]. The stepwise multivariate regression analysis performed in the entire population showed that HbA1c (but not age, gender, or BMI) independently predicted the gene expression of HIF-1α [15]. Our results may suggest the potential effect that the improvement of metabolic control can exert on the induced expression of HIF-1α gene in patients with T2DM.

Our next findings have shown a significant decrease in mTOR gene expression in blood leukocytes of patients with T2DM. It is known that mTOR and its related signaling pathways can govern cellular metabolism, stem cell maintenance and viability, insulin secretion and resistance, apoptosis, autophagy, and pancreatic β-cell mass and function [17-19]. In regards to the OS developing, the established mTOR inhibition can have a dual role. On the one hand, a reduction in mTOR activity promotes the induction of both apoptosis and autophagy against oxidized low-density lipoproteins which lead to OS in human retinal capillary pericytes and pancreatic β-cells [19]. Moreover, increased AMPK (AMP-activated protein kinase) activity that occurs during mTOR inhibition can reduce insulin resistance and OS developing in multiple tissues through the activation of autophagy in experimental models of T2DM [35]. On the other hand, a reduction in mTOR activity can foster OS injury of diabetic tissues because of a tight relationship between mTOR and such metabolic regulators as growth factors, erythropoietin (EPO), AMPK, protein kinase B (Akt), phosphoinositide 3-kinase (PI_3-K), silent mating type information regulation 2 homolog 1(SIRT1), etc. In particular, EPO is critical in mTOR signaling [19]. EPO uses mTOR for limitation OS in Schwann cells, retinal progenitor cells, and hippocampus-derived neuronal cells [26, 37, 38]. EPO modulates a specific level of AMPK and mTOR activity to alleviate detrimental effects of OS [39]. Thus the established marked decrease in mTOR gene expression would also mirror an increase in oxidative stress developing during T2DM.

So, we suggest that the established changes in HIF-1α and mTOR genes expression in blood of patients with T2DM may be estimated as protective reactions against OS developing. The question is whether these reactions represent the adaptive defensive responses developing in the prolonged course of T2DM or could be due to the use of antidiabetic therapies. There is no sufficient information to date about the effects of pharmacologic agents used in the treatment of our patients (oral glucose-lowering medications, statins, angiotensin-receptor blockers or inhibitors of angiotensin-converting enzyme) either on the HIF-1α gene expression level or on the transcriptional activity of HIF-1α. But, in regard to mTOR, there was shown that Metformin has mitigating effect on ROS production and regulates very markedly mTORC1 activity [18]. In any case, the defensive reactions of HIF-1 α and mTOR genes expression are not sufficient to protect cells completely against OS developing under T2DM. These reactions are in need of the support of pharmacologic targeting which could be the part of potential therapeutic strategy for antioxidant treatment in T2DM.

Conclusions. Patients with T2DM showed a significantly increased plasma concentration of oxidative stress reliable biomarkers – protein carbonyls (the products of oxidative protein modification) and TBARS (secondary products of lipid peroxidation) as well as a significant rise in the production of H_2O_2 in erythrocytes. The established rise in SOD and catalase activities in blood of patients with T2DM can be estimated as a compensatory reaction to the increased production of ROS – superoxide anion and

hydroperoxide. Deterioration of glutathione antioxidant system (a decrease in GSH level and GP_x activity) in erythrocytes of patients with diabetes may serve as a mechanism of OS development connecting with the intracellular thiol redox state impairment and intensification of prooxidant processes. The established tendency to an increase in HIF-1α gene expression in leukocytes of diabetic patients may represent a mechanism of minimization of oxidative cell damage. A blunted level of HIF-1α gene expression in T2DM could be connected with the inhibited HIF-1α signaling which may accelerate OS developing. The established marked inhibition of mTOR gene expression in leukocytes of patients with T2DM may serve as a protective mechanism which counteracts OS developing, mainly through induction of autophagy against oxidized low-density lipoproteins, using different signaling metabolic pathways.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The study was supported by budget funding from Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine: "Molecular-genetic mechanisms of hypoxic influence on inflammation and metabolic disturbances" № 01191U103909.

ОКСИДАТИВНИЙ СТРЕС У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ: МОДУЛЯЦІЯ ЕКСПРЕСІЇ ГЕНІВ HIF-1A ТА mTOR

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Вивчено біохімічні та генетичні механізми розвитку оксидативного стресу (ОС) в крові хворих на цукровий діабет 2 типу (ЦДТ2). Двадцять хворих на ЦДТ2 і 10 здорових осіб (контроль) брали участь у дослідженні. Як біомаркери розвитку ОС у плазмі та еритроцитах крові вимірювалися наступні показники: рівень ліпідної пероксидації (за утворенням активних продуктів тіобарбітурової кислоти, ТБКАП), рівень оксидативної модифікації протеїнів (за концентрацією протеїнових карбонілів) та рівень продукції пероксиду водню. Визначали активність ензимів супероксиддисмутази (СОД), каталази та глутатіонпероксидази (GP_x), а також рівня відновленого глутатіону (GSH) в плазмі та еритроцитах. Досліджено експресію генів ключових регуляторів кисневого та метаболічного гомеостазу (HIF-1α і mTOR) у лейкоцитах крові. Встановлено значне підвищення вмісту ТБКАП і протеїнових карбонілів у плазмі крові, а також ріст утворення H_2O_2 в еритроцитах хворих на ЦДТ2 у порівнянні з аналогічними показниками контрольної групи. Хворі на ЦДТ2 демонстрували зростання активності СОД і каталази в плазмі, а також значне зменшення концентрації GSH та активності GP_x в еритроцитах у порівнянні з контролем. Встановлене виражене пригнічення експресії гена mTOR і тенденція до підвищення експресії гена HIF-1α в лейкоцитах хворих на ЦДТ2 можуть слугувати захисним механізмом, який протидіє розвитку ОС та окислювальному пошкодженню клітин.

Ключові слова: оксидативний стрес, HIF-1α, mTOR, цукровий діабет 2 типу.

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