

Ischemia-modified albumin level in type 2 diabetes mellitus – Preliminary report

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Abstract. *Aim:* The main goal of the present study was the evaluation of ischemia-modified albumin (IMA) in patients with type 2 diabetes mellitus and estimation of its connection with vascular complications, glycemic control, hypertension, dyslipidemia and obesity.

Methods: In 76 diabetic patients and 25 control subjects, a plasma level of IMA by manually performed, spectrophotometric Co(II)-albumin binding assay was determined. Other parameters such as glucose, fructosamine, HbA_{1c}, total cholesterol and its fractions (HDL, LDL), triglycerides were estimated by routine methods.

Results: Diabetic patients had significantly higher level of IMA in comparison with control subjects. There were not significant differences between groups with various states of vascular complications although the lowest concentration of IMA was observed in patients with microangiopathy. Patients with poor glycemic control had higher IMA level in comparison with these with good glycemic control. Significant correlation was observed between IMA and HbA_{1c}. Among the risk factors, only blood pressure and LDL showed a weak relationship with IMA level.

Conclusions: Our results revealed, for the first time, higher level of IMA in diabetic patients which confirms that it may be of non-cardiac origin. We can suggest that the albumin molecule in plasma of diabetic patients is modified in the chronic hypoxia conditions provoked mainly by hyperglycemia and oxidative stress in diabetes.

1. Introduction

Recent literature reports show large interest in new biochemical marker – ischemia-modified albumin (IMA) for detection of myocardial injury. Special attention is focused on estimation of IMA test for the diagnosis and evaluation of myocardial ischemia as well as others acute coronary syndrome in emergency patients. Because ischemia, and the resulting biochemical changes, can occur in any vessel, the specificity of IMA for cardiac ischemia is unclear [1,24,28].

Myocardial ischemia and accompanying hypoxia induced the structural modifications of human serum albumin (HSA). HSA performs many essential functions

in the organism, among the others direct protective effect on oxidative stress. This molecule represents one of the circulating antioxidant in plasma and plays a vital role in the efficient antioxidant defense of the organism [4,8].

In vivo study revealed that serum albumin of individuals with myocardial ischemia exhibits reduction in its inherent affinity for metal transition ions such as Co(II), Ni(II) and Cu(II) compared to non-ischemic ones. The biochemical mechanism appears to be reversible. It causes ions-albumin binding alteration and it is not yet fully understood. *In vivo* studies show, that the molecule of albumin changes the ability of the first three amino acids N-Asp-Ala-His to bind free metal ions, after modifications in hypoxia conditions [5,12]. This abnormal molecule of HSA is known as Ischemia-Modified Albumin (IMA) and it is measured by the spectrophotometric Co(II)-albumin binding assay. The concentration of IMA is determined by addition of a

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known amount of exogenous Co(II) to a serum sample and measurement of unbound Co(II) by colorimetric assay using dithiothreitol (DTT). An inverse relationship exists between the amount of albumin-bound cobalt and the intensity of the color formation, reported in absorbance units (ABSU) [2,4]. IMA is a registered trade mark purchased by Inverness Medical/Unipath Ltd.

There are several data on IMA in patients with different states with ischemia of non-cardiac origin such as systemic sclerosis [7,21], peripheral vascular disease [26], skeletal muscle ischemia during arthroscopic knee surgery and exercise induced [19,22,24,26,31,32] but no one concerns diabetes. Hyperglycemia and oxidative stress can induce chronic ischemia in diabetic patients. It could lead to necrosis of different tissues [7,17,20].

The main goal of the present study was the assessment of ischemia-modified albumin concentration by manually performed colorimetric assay described by Bar-Or et al. [4] in type 2 diabetes mellitus patients. We also wanted to check if IMA level in diabetics is related to vascular complications as well as different risk factors of this disease, such as poor glycemic control, hypertension, obesity, dyslipidemia.

2. Material and methods

Seventy-six patients with type 2 diabetes mellitus, treated at the Clinic of Angiology, Hypertension and Diabetology of Wrocław Medical University, were studied. They were informed about the aim of these investigations and gave their permission to enter this study. We got the agreement of The Local Bioethics Committee of Wrocław Medical University on conducting these researches. All patients were in stable clinical status without signs of acute infections and acute ischemia. A control group consisted of 25 healthy adults without neither inflammatory states nor abnormalities in lipids and carbohydrate metabolism, in routine medical check-ups. Biological and physical characterization of these two groups is given in Table 1.

Vascular late complications in diabetic patients had been recognized on the basis of medical and biochemical investigations. Nineteen of diabetics had microangiopathy (retinopathy, nephropathy, neuropathy, diabetic foot), thirty-one patients had macroangiopathy (peripheral arteriosclerosis, coronary disease, myocardial infarction and stroke in the history of disease), and twenty-six patients had both types of diabetic complica-

tions. Patients of the two latest groups, with macroangiopathy or both types of complications (micro- and macroangiopathy), have myocardial ischemia or necrosis incidents recognized in their history of disease. Twenty-five patients had fasting plasma glucose concentration below 8.5 mmol/l (good short-term glycemic state), thirty-six patients had level of fructosamine below 3.0 mmol/l (good middle-term glycemic state) and twenty-six patients had level of glycated hemoglobin below 7% HbA_{1c} (good long-term glycemic state). Sixteen patients had normal body mass index (BMI lower than 24.9), twenty-one patients were overweight (BMI between 25.0 and 29.9) and remaining thirty-nine patients were obese (BMI higher than 30). There were fifty-one percent of diabetics with hypertension, almost forty percent with hypercholesterolemia, twenty-nine percent with hypercholesterolemia-LDL and fifty-four percent of patients with hypertriglyceridemia.

Venous blood samples were drawn in a fasting state to tubes containing heparin (250 units/ml). Blood concentration of glycated hemoglobin (HbA_{1c}) was determined and then the samples were centrifuged. Plasma was immediately frozen and stored at -85°C until the investigations were held (no longer than three months). To access the ability of binding exogenous cobalt Co(II) to human albumin in plasma we used a manual colorimetric assay described by Bar-Or et al. [4]. Briefly, fifty μl water solution of 0.1% cobalt chloride ($\text{CoCl}_2\cdot 6\text{H}_2\text{O}$) was added to 200 μl of plasma, gently mixed and after 10 min (for adequate cobalt-albumin binding), the 50 μl of dithiothreitol (DTT) solution (1.5 mg/ml H_2O) was added as a colorizing agent and the reaction was quenched two minutes later by adding 1.0 ml of 0.9 % NaCl. Colour development with DTT was measured spectrophotometrically (SPECOL 11) at 470 nm in comparison with a plasma-cobalt blank without DTT and reported in absorbance units (ABSU). Each sample was measured in duplicate and the mean value was reported. For applied manual technique of IMA determination the intra-assay and inter-assay CV was 2.31% and 4.21%, respectively.

Plasma glucose was determined by simple, colorimetric, enzymatic method in ALCYON 300 Abbott using Cormay diagnostic kit. Assay precision within run and run to run as well as sensitivity were: CV 2.2%, CV 2.1%, 0.03 mmol/l, respectively. Glycated hemoglobin (HbA_{1c}) was determined from hemolysates, prepared on the board of the COBAS[®] chemistry system from the whole blood, by a latex enhanced turbidimetric immunoassay and measured at 550 nm. Total Hb was determined by the colorimetric cyanide-free alkaline

Table 1
Characteristic and clinical values (mean \pm standard deviation) of control subjects and patients with type 2 diabetes mellitus

	Control subjects	Type 2 diabetes mellitus
Number	25	76
Sex (F/M.)	19/6	65/11
Age (years)	56.85 \pm 18.60	64.67 \pm 11.72
Disease duration (years)	–	14.95 \pm 8.63
Mean blood pressure (systolic/diastolic; mmHg)	128/75 \pm 11/8	141/76 \pm 14/10
BMI (kg/m ²)	25.38 \pm 4.37	30.00 \pm 5.52**
FPG (mmol/l)	4.61 \pm 0.17	8.57 \pm 2.13***
Fructosamine (mmol/l)	2.03 \pm 0.62	3.89 \pm 0.80**
HbA _{1c} (%)	4.30 \pm 0.45	9.03 \pm 1.72***
Total cholesterol (mg/dl)	195.98 \pm 53.32	219.79 \pm 52.18
HDL-cholesterol (mg/dl)	58.01 \pm 15.21	62.04 \pm 22.05
LDL-cholesterol (mg/dl)	107.80 \pm 29.52	126.52 \pm 39.49*
Triglycerides (mg/dl)	129.62 (40.800–182.10)	169.80** (60.60–538.30)
Albumin (g/l)	51.11 \pm 5.54	54.01 \pm 6.91

Comparison to healthy subjects: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

BMI – body mass index; FPG – fasting plasma glucose.

Triglycerides are given as median and range (min – max).

hematin method. The final HbA_{1c} test result was calculated from the HbA_{1c}/Hb ratio. Assay precision within run and run to run as well as sensitivity were: CV 2.6%, CV 1.8%, 0.76 μ mol/l of hemoglobin and 0.78 μ mol/l of HbA_{1c}, respectively. Total cholesterol and its fractions (cholesterol-HDL and -LDL) as well as triglycerides were determined using routine clinical assays.

Diagnostic assay data were expressed as mean values \pm standard deviation (SD). Statistical analysis was performed with Statistica PL for Windows, version 5. Results were analysed by U Mann-Whitney's test and/or by analysis of variance (ANOVA). Obtained p value which was less than 0.05 was considered as statistically significant. We also used Spearman test or multiple linear regression analysis to show any correlation between those data.

3. Results

Level of ischemia-modified albumin (expressed as ABSU units \pm SD) in plasma samples of control subjects and patients with diabetes type 2 are shown in Table 2. Diabetic patients had significantly ($p < 0.001$) higher IMA level (about 75%) than the control group (0.561 ABSU vs 0.328 ABSU) what indicates essential reduced binding capacity of albumin molecule to exogenous cobalt.

The lowest value of IMA (0.548 ABSU) was observed in diabetics with microangiopathy but there were

not significant differences between groups with various states of vascular complications.

Table 3 shows the plasma levels of IMA in the groups of diabetic patients with good and poor glycemic control described by fasting glucose plasma concentration (short-term glycemic control), fructosamine (middle-term glycemic control) as well as glycated hemoglobin (long-term glycemic control). The IMA value was higher in all groups with poor glycemic control in comparison with these with good controlled diabetes. This increase was revealed only in patients with poor long-term glycemic state (HbA_{1c} higher than 7%) statistically significant ($p < 0.05$). Significant correlation ($r = 0.42$, $p < 0.05$) was observed only between glycated hemoglobin and IMA level. Relationships among remaining parameters, fasting plasma glucose and fructosamine, were not statistically significant ($r = 0.16$ and $r = 0.28$, respectively).

We also analysed the ability of human albumin to exogenous cobalt binding in groups of patients clustered on the basis of vascular complications risk factors like: hypertension, lipid disorders and obesity. Patients with hypertension as well as hypercholesterolemia-LDL had slightly higher IMA level but there were no statistically significant differences among selected groups (Table 4). There was observed a weak but not statistically significant relationship, only between value of ABSU units and blood pressure ($r = 0.23$) and LDL-cholesterol ($r = 0.25$).

There was not statistically significant relationship between value of ABSU units and sex, age and disease duration (date no shown).

Table 2
Plasma level of IMA measured by Cobalt-HSA binding test, expressed as absorbance units (ABSU), in healthy people and patients with type 2 diabetes mellitus and in groups with different vascular late complications

Groups	Number of subjects	ABSU \pm SD
Healthy people	25	0.328 \pm 0.095
Patients with type 2 diabetes mellitus	76	0.561 \pm 0.107***
Groups of patients with diabetic complications		
microangiopathy	19	0.548 \pm 0.104
macroangiopathy	31	0.561 \pm 0.139
micro- and macroangiopathy	26	0.575 \pm 0.125

(***) $p < 0.001$ comparison between diabetic patients and healthy people.

Table 3
Plasma level of IMA measured by Cobalt-HSA binding test, expressed as absorbance units (ABSU), in groups of diabetic patients with different states of glycemic control

Parameters of glycemic control	Groups with different state of glycemic control	Number of subjects	ABSU \pm SD
Short term (glucose)	good (≤ 8.5 mmol/l)	25	0.523 \pm 0.124
	poor (> 8.5 mmol/l)	51	0.571 \pm 0.109
Middle term (fructosamine)	good (≤ 3.0 mmol/l)	36	0.535 \pm 0.133
	poor (> 3.0 mmol/l)	40	0.574 \pm 0.095
Long term (HbA ₁)	good ($\leq 7\%$)	26	0.493 \pm 0.129
	poor ($> 7\%$)	50	0.563 \pm 0.109*

(*) $p < 0.05$ comparison between groups of patients with poor and good glycemic control.

Table 4
Plasma level of IMA measured by Cobalt-HSA binding test, expressed as absorbance units (ABSU), in groups of diabetic patients clustered on the basis of different risk factors of angiopathy

Groups	Number of subjects	ABSU \pm SD	
Blood pressure	Normotension ($\leq 85/135$ mmHg)	35	0.542 \pm 0.128
	Hypertension ($> 85/135$ mmHg)	41	0.578 \pm 0.114
Total cholesterol	Normal level (≤ 200 mg/dl)	46	0.554 \pm 0.124
	Hypercholesterolemia (> 200 mg/dl)	30	0.567 \pm 0.140
LDL-cholesterol	Normal level (< 85 mg/dl)	54	0.547 \pm 0.100
	Hypercholesterolemia-LDL (> 85 mg/dl)	22	0.571 \pm 0.136
Triglycerides	Normal level (≤ 135 mg/dl)	37	0.553 \pm 0.119
	Hypertriglyceridemia (> 135 mg/dl)	39	0.547 \pm 0.107
BMI	Normal (≤ 24.9 kg/m ²)	16	0.562 \pm 0.065
	Overweigh ($> 25.0 \leq 29.9$ kg/m ²)	21	0.570 \pm 0.098
	Obese (≥ 30 kg/m ²)	39	0.569 \pm 0.117

4. Discussion

Ischemia-modified albumin has been studied primarily in selected populations to display myocardial in-

volvement only in the absence of confounding clinical conditions. IMA increases also in brain ischemia, end-stage renal disease, liver disease, some neoplasms, infections as well as in patients with peripheral vas-

cular diseases and exercise-induced skeletal muscle ischemia [3,8,11]. Recent studies indicate that structural modifications of albumin can occur as the result of endothelial and extracellular hypoxia, acidosis, reduced oxygen tension, various ion-pump disruptions, and generation of reactive oxygen species (ROS) [2,11,12].

Reactive oxygen species are well known as a factor responsible for chemical and molecular damage of many biological molecules (proteins, lipids, carbohydrates, DNA, nucleotides) and cell membrane structure. Oxidative stress is important in variety of physiological (e.g., aging) and pathological (e.g., atherosclerosis, diabetes) processes [15,30]. In diabetic patients hyperglycemia, via several mechanisms (glucose autooxidation, stimulation of the polyol pathway, imbalance between the amount of reduced and oxidized coenzymes forms, nonenzymatic glycation and formation advanced glycation end-products-AGEs), leads to multiple biochemical sequel resulting in oxidative stress. It plays significant role in pancreatic islets destruction in diabetes type 2 and leads to its late complications [6,16,25]. This also causes the oxidative protein damage, formation of advanced oxidation protein products (AOPP) and probably IMA [9,14,29].

Although the definitive and precise mechanism for IMA production *in vivo* is unknown as yet, it appears to be related to the generation of ROS that modifies metal binding domains of albumin. Both indirect and direct evidence supports this concept. Cobalt chloride is a well-established chemical inducer of hypoxia-like responses, such as erythropoiesis and angiogenesis *in vivo*, likely involving an increased DNA binding activity of hypoxia-inducible factor-1 α (HIF-1 α) to its target genomic sequences. It has been speculated that cobalt might stabilize HIF-1 α through generation of ROS by a nonenzymatic, mitochondrial mechanism. Under normoxic conditions, the main mediator HIF-1 α is rapidly degraded by the proteasome [18]. Oxidative stress and hyperglycemia are also well recognized pathogenic processes for atherosclerosis and cardiovascular disease. Hyperglycemia stress has been observed previously in up to two thirds of patients with an acute myocardial infarction and was found to be associated with amplified inflammatory immune reactions. Recently IMA is suggested for early detection of ischemia also in different arterial regions [3,17].

In relation with the primary aim of our study which was mentioned above, we wanted to check if IMA levels, expressed as ABSU units, increases in diabetes type 2 mellitus and if there are any differences be-

tween patients with vascular complications (micro- and macroangiopathy). The secondary aim was to determine how glycemia influence the albumin modification. Additionally the relationship of the IMA concentration with other conventional risk factors associated with diabetes, including obesity, blood pressure, and incorrect lipid profile was investigated. We estimated IMA levels in plasma of diabetic patients and control subjects using the manual method. In the manufactures kit for ACB assay, using the Cobas MIRA[®] Plus instrument, serum sample is advised [13]. Before starting the study we determined level of IMA in the samples of serum and plasma of the same diabetic patients (twelve persons) and we did not reveal any statistically significant differences between these two biological materials. We used plasma in order to simultaneously isolate polymorphonuclear leukocytes from the same blood sample for another researches (date not shown).

We observed about 75% higher level of ischemia-modified albumin in plasma of type 2 diabetic patients in relation to control subjects. This confirms previous, as well as our observations, that increased ROS generation, provoked by hyperglycemia, can cause oxidative protein damage. In the case of albumin decreasing ability to exogenous cobalt binding in plasma has been reported [8,10,12]. We observed higher value of IMA in the groups with micro- and macroangiopathy in comparison with the healthy people but there was no significant difference between the patients in these groups with angiopathy. Such results suggest that albumin modification may occur in the early stage of the disease and take part in the pathogenesis of diabetes. IMA may indicate underlying subclinical disease or vascular dysfunction, what suggested also Borderie et al. [7]. Previously, we reported that diabetes is connected with excessive protein oxidative stress, associated with higher AOPP and carbonyl (CO) groups concentration as well as lowered level of thiol (SH) groups and total radical-trapping antioxidant parameter (TRAP). The highest plasma AOPP level in diabetics with macroangiopathy, significantly different from that in microangiopathy as well as both types of angiopathy was observed [23]. In contrast, the decrease of IMA concentration was not significant in patients with microangiopathy in comparison with macroangiopathy in the present work.

The IMA, increasing during ischemia-reperfusion, affects any organ and cannot be considered a specific cardiac marker in diseases associated with oxidative stress. The high concentration of IMA 24–48 h after endurance exercise was observed as well as in patients undergoing major uncomplicated orthopedic surgery, with

peripheral vascular disease and leg claudications [20, 24,26]. Many reports pointed out the role of peripheral vascular disease as an independent predictor of mortality in patients with coronary artery disease [20]. Hyperglycemia and oxidative stress in diabetic patients are well-recognised contributors of pathogenic process for endothelium damage, atherosclerosis and cardiovascular disease [10]. Controlling hyperglycemia after acute myocardial infarction and more generally in the critically ill is a way to reduce mortality of these patients [17].

In diabetic patients circulating albumin is still exposed to continuous higher glycemia and oxidative stress. For this reason we checked the IMA level in groups of patients with different level of diabetes compensation, described by fasting glucose plasma concentration, fructosamine as well as glycated hemoglobin. In all groups with poor glycemic compensation higher concentration of IMA was observed in comparison with these with good compensated diabetes. Only in the patients with HbA_{1c} higher than 7% significant correlation between IMA and HbA_{1c} was found. The binding of glucose to albumin typically occurs *in vivo* in healthy people and is known to involve the nonenzymatic covalent attachment of glucose to a lysine side chain but increases between two- to threefold in hyperglycemia. Thus it is possible that patients with more severe course of disease (poorly controlled diabetes compared to well controlled diabetes as well as to healthy people) could occur greater free radical production, leading to higher IMA concentration. Moreover, diabetic patients exhibit elevated levels of iron and copper ions that, in the presence of glycated proteins, have been shown *in vitro* to generate ROS [5]. This indicates that IMA level should be considered in the context of well and bad compensated diabetes.

Our current findings, although preliminary, show that chronic oxidative stress provoked by hyperglycemia, in diabetic patient causes the decrease of albumin ability to exogenous cobalt binding. It supports the hypotheses that the rise in IMA level may be also of non-cardiac origin. Our diabetic patients had no recent episodes of chest pain, acute heart failure or unstable angina pectoris, but they had these incidences in the their disease history. We observed the highest value of IMA in patients with micro- and macroangiopathy but the ones with diabetes microangiopathy had the lowest value of IMA. We also reveal higher IMA concentrations in diabetic patients with poor long-term glycemic control, probably with acidosis, hypertension as well as hypercholesterolemia-LDL compared with baseline.

This may have implications regarding the ability of IMA to detect myocardial ischemia in diabetics. Recently Roy et al. [27] suggested and revealed the role of reactive oxygen species, such as superoxide (O_2^-) and hydroxyl (OH) radicals, generated during myocardial ischemia-reperfusion, on the modification of the N-terminus of albumin and formation of the IMA.

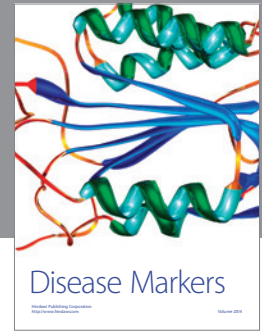
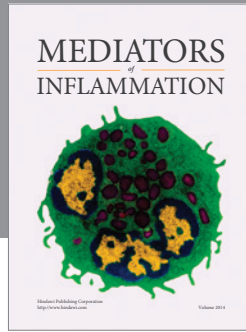
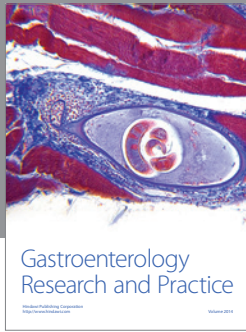
A positive IMA value could also help to identify higher risk individuals, suffering from local or systemic hypoxic conditions, as acute ischemic stroke, peripheral vascular disease, systemic sclerosis, peripheral vascular intervention, exercise-induced calf-muscle ischemia, end-stage renal disease.

We suggest that measurements of IMA in diabetic patients type 2 can be helpful in their diagnosis and monitoring of the course of disease, especially with different states of renal disorders, what will be of an object of our future researches. But first of all we think that measurements of IMA can be very important factor in correct diagnosis and classification of patients with co-existed diabetes who come to emergency department with chest pain.

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