

## BIOMARKERS FOR DIABETES COMPLICATIONS: THE RESULTS OF SEVERAL CLINICAL STUDIES

### BIOMARKERI KOMPLIKACIJA U DIJABETESU: REZULTATI NEKOLIKO KLINIČKIH STUDIJA

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**Summary:** Diabetes is a common metabolic disorder. Its microvascular and macrovascular complications contribute to death, disabilities, and reduction in life expectancy in diabetes. It is a costly disease, and affects not only the patient and family, but also the public health, communities and society. It takes an increasing proportion of the national health care expenditure. The prevention of the development of diabetes and its complications is a major concern. Biomarkers have been investigated for understanding the mechanisms of the development and progression of diabetic complications. In this paper, the biomarkers which are recommended in the clinical practice and laboratory medicine guidelines, and which have been investigated for prediction or diagnosis of diabetes complications, have been reviewed. The results of several clinical studies will be summarized.

**Keywords:** diabetes, biomarkers, clinical studies

### Introduction

Diabetes mellitus is a complex metabolic disorder and one of the main chronic diseases worldwide. The number of people with diabetes is estimated at 285 million in 2010, and it is expected to be over 438 million by the year 2030 (1). Close to four million deaths in the 20–79 age group may be attributable to diabetes in 2010, accounting for 6.8% of the global all-cause mortality in this age group (2). Besides the impact on the patients' quality of life, the microvascular (diabetic retinopathy–DR, nephropathy–DN, neuropathy) and macrovascular complications (coronary heart diseases,

**Kratak sadržaj:** Dijabetes predstavlja čest metabolički poremećaj, čije mikrovaskularne i makrovaskularne komplikacije doprinose smrti, invaliditetu i skraćenju očekivanog životnog veka kod obolelih. Ova bolest podrazumeva velike troškove a pored pacijenta i njegove porodice po- gađa i javno zdravlje, zajednice i društvo. Dijabetes zahteva sve veći deo nacionalnih troškova zdravstva. Prevencija raz- voja dijabetesa i njegovih komplikacija je važan problem. U cilju razumevanja mehanizama razvoja i progresije kompli- kacija u dijabetesu vrši se istraživanje biomarkera. U ovom radu dat je pregled biomarkera koji se preporučuju u kli- ničkoj praksi i pravilnicima za laboratorijsku medicinu i koji su istraživani radi predikcije ili dijagnostikovanja kompli- kacija u dijabetesu. U sažetom obliku su prikazani rezultati nekoliko kliničkih studija.

**Ključne reči:** dijabetes, biomarkeri, kliničke studije

peripheral artery diseases, and stroke) of diabetes also in- crease the national health care expenditure. Estimated global health care expenditures to treat and prevent diabetes and its complications are expected to total at least 376 billion US Dollars (USD) in 2010. By 2030, this number is projected to exceed some 490 billion USD (3). Globally, diabetes is likely to be the fifth leading cause of death (4).

Prevention of diabetes and its complications, early detection of disease stages, and therapeutics that would act in the presence of hyperglycemia to prevent, delay or reverse the complications are the major con- cerns. Biomarkers are studied for understanding the mechanisms of hyperglycemia-caused metabolic abnormalities (5, 6) such as polyol pathway activation, non-enzymatic glycosylation/Maillard reaction, activa- tion of protein kinase C (PKC), altered gene expression, and growth factor activation. These include biomarkers of inflammation (7, 8), advanced glycation (9–12),

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endothelial dysfunction, oxidative stress and antioxidant mechanisms (10, 11, 13–15), hemostasis/thrombosis, cellular adhesion molecules, mitochondrial dysfunction, and the activation in the PKC signaling pathway, lipid status (8), and microangiopathies that cause organ damage (9, 16–19).

Besides the standard laboratory techniques, advanced technologies such as genomics, proteomics (20–26), metabolomics (27), transcriptomics (28), lipidomics (29–31) and glycomics (32) have been used to identify biomarkers.

Although uncontrolled hyperglycemia-related tissue damage is the primary cause of diabetic complications, the course of complications may be altered by genetic and environmental factors, and therefore the complications are not developed to the same degree in all patients. In this context, the genetic basis of diabetes complications is also being investigated (33–42).

The pathogenesis of diabetes complications is complex and multifactorial, has extensive implications, and leads to multiorgan failure. There is an established heterogeneity in the determinants of the risk of diabetes

complications. The heterogeneity leads to consideration of the personalized approach to diagnostic and treatment strategies of diabetes and its complications (43–45). The spectrum of information that can guide personalized decisions on diabetes care also includes individual behavioral and clinical phenotypic features, standard clinical laboratory findings, and gene sequences and other molecular markers. All techniques have been used for the identification of biomarkers.

We should also keep in mind that the occurrence and progress of diabetes complications is not influenced only by hyperglycemia and related metabolic abnormalities, but also by the presence of nonglycemic risk factors such as hypertension, dyslipidemia (46), as well as age, duration of diabetes, and obesity.

### **Biomarkers recommended for management of diabetes and prevention of its complications**

The biomarkers related to laboratory measurements, and recommended for the assessment of diabetes complications are summarized in *Table I*. As seen in

**Table I** Biomarkers related laboratory measurements recommended for the prevention, prediction and/or diagnosis of diabetes complications in the guidelines.

Guideline	Recommended Biomarkers	Recommendations/Comments/Evidence/Conclusions
NACB 2010 (47)	<ul style="list-style-type: none"> <li>• Glucose</li> <li>• Glycated hemoglobin (HbA1c)</li> <li>• Ketone testing</li> <li>• Urinary albumin excretion rate and uAlb:creat ratio</li> </ul>	There is a direct relationship between chronic hyperglycemia and the risk of renal, retinal and neurological complications. The correlation has been reported in epidemiologic and clinical studies for both types of diabetes. – HbA1c is measured in all patients with diabetes to document their degree of glycemic control, and used both as an index of mean glycemia and as a measure of risk of diabetes complications. – Ketone testing: for diagnosing DKA. – Microalbuminuria is a well established cardiovascular risk marker, whose increases over time to macroalbuminuria are associated with increased risk for the development of end-stage renal disease.
ADA 2010 (46)	<ul style="list-style-type: none"> <li>• Blood glucose</li> <li>• HbA1c</li> <li>• Urinary Alb excretion/ACR</li> <li>• Serum creatinine</li> <li>• Lipid profile (LDL-Chol, HDL-Chol, TG)</li> </ul>	<b>Components of the comprehensive diabetes evaluation: Laboratory evaluation</b> – HbA1c, if results not available within past 2–3 months; and if not performed/available within past year. – Fasting lipid profile, including total, LDL- and HDL cholesterol and triglycerides. – Liver function tests. – Test for urine albumin excretion with spot urine albumin/creatinine ratio. – Serum creatinine and calculated GFR. – TSH in type 1 diabetes, dyslipidemia, or women over age 50.
NHMRC 2009 (48)	<ul style="list-style-type: none"> <li>• Glucose</li> <li>• Glycated hemoglobin (HbA1c)</li> <li>• Urinary albumin excretion rate and uAlb:creat ratio</li> <li>• eGFR (Cockcroft-Gault and MDRD)</li> <li>• TGF-beta in urine</li> </ul>	– Microalbuminuria is a key predictor for the development of CKD in people with type 2 diabetes, however CKD may develop in the absence of abnormalities in albumin excretion (Level II – Prognosis). – AER and ACR are the most common and reliable methods to assess albuminuria based on sensitivity and specificity, however both methods are subject to high intra-individual variability so that repeated tests are needed to confirm the diagnosis (Level III – Diagnostic Accuracy). – Estimation of GFR (eGFR) based on serum creatinine is a pragmatic, clinically relevant approach to assessing kidney function in people with type 2 diabetes (Level III – Diagnostic Accuracy).

Table I, the recommended biomarkers for assessing the complications of diabetes are few, and yet none of them is relevant for the prediction and diagnosis of diabetes complications.

Although HbA1c is a good marker for the determination of mean glycaemia, and microalbuminuria is considered to be a predictor of cardiovascular disease, and if it increases gradually to macroalbuminuria, microalbuminuria is also associated with end-stage renal disease, it is currently impossible to reliably predict when and which diabetic patients will develop retinopathy, nephropathy or neuropathy. Biomarkers are urgently needed for the early pre-clinical stages through the late organ failure stages of diabetes complications.

### Molecules investigated for determination of progression, prediction and/or diagnosis of diabetic complications

As seen in Table II, there are lots of molecules that are associated with the metabolic abnormalities which are caused by hyperglycemia and have been studied

for the prediction and/or diagnosis of diabetes complications.

Matheson et al. (19) reviewed urinary biomarkers that may be used to monitor the development and progression of diabetes and its complications. Their conclusion is that biomarkers of renal dysfunction (such as **transferrin, type IV collagen and N-acetyl- $\beta$ -D-glucosaminidase**) may prove to be more sensitive than urinary albumin in the detection of incipient nephropathy and risk assessment of cardiovascular disease. Inflammatory markers including **orosomucoid, tumour necrosis factor- $\beta$ , transforming growth factor- $\beta$ , vascular endothelial growth factor and monocyte chemoattractant protein-1**, as well as oxidative stress markers such as **8-hydroxy-2 deoxyguanosine** may also be useful biomarkers for the diagnosis or monitoring of diabetic complications, particularly kidney disease.

Ameur et al. (26) have reviewed the proteomics studies devoted to DN biomarkers discovery between 2004 and 2009 by dividing them into those focused on diagnosis and those that focused on prediction. They found 34 urinary proteins to be upregulated and 34 downregulated. Riaz et al. (49) identified **transthy-**

**Table II** Molecules that are associated with the metabolic abnormalities which are caused by hyperglycemia.

<p><b>Urinary biomarkers of renal damage classified by type of diabetes and by diabetic complication investigated in the study: type 1 diabetes (T1), type 2 diabetes (T2), nephropathy (DN), retinopathy (DR) or cardiovascular disease/macrovascular disease (CVD/MVD):</b> alanine aminopeptidase (T1,T2, DN, DR), albumin (T1,T2, DN, DR, CVD/MVD), alkaline phosphatase (T1,T2, DN), <math>\alpha</math>1-microglobulin (T1,T2, DN); <math>\beta</math>2-Glycoprotein- 1/apolipoprotein H (T1,T2, DN), <math>\beta</math>2-Microglobulin (T1,T2, DN, CVD/MVD); <math>\beta</math>-Ig-h3 (T2, DN), cathepsin B (T1,T2, D), ceruloplasmin (T2, DN), dipeptidyl aminopeptidase IV (T2, DN), epidermal growth factor (T1,T2, DN, DR), fibronectin (T1,T2, DN), <math>\gamma</math>-glutamyl-transferase (T1,T2, DN, DR), glycosaminoglycan (T1,T2, DN, DR), immunoglobulin-free light chains (T1,T2, DN, DR), immunoglobulin G (T1,T2, DN, DR), laminin (T1,T2, DN, DR), lipocalin-type prostaglandin D synthase (T2, DN, CVD/MVD), N-acetyl-<math>\beta</math>-D-glucosaminidase (T1,T2, DN, DR; CVD/MVD), retinol-binding protein ((T1,T2, DN, DR; CVD/MVD), Tamm – Horsfall protein/uromodulin (T1,T2, DN), transferrin (T1,T2, DN, DR; CVD/MVD), type IV collagen (T1,T2, DN, DR; CVD/MVD) (19).</p>
<p><b>Urinary proteins (or their fragments) found associated with renal damage in the context of diabetes, and discovered by proteomic approaches (e.g. 2D-GE and MALDI-MS/MS) or by profiling methods (e.g. SELDI-TOF-MS).</b>  <b>Downregulated proteins:</b> <math>\alpha</math>1-microglobulin/bikunin precursor (AMBP), apolipoprotein A-I, apolipoprotein CIII, apolipoprotein E, collagen <math>\alpha</math>-6 (IV), collagen <math>\alpha</math>-1 (IV), collagen <math>\alpha</math>-1 (V), collagen <math>\alpha</math>-1 (I), collagen <math>\alpha</math>-1 (III), collagen <math>\alpha</math>-2 (I), complement component C4 A, complement factor H-related 1, complement factor I light chain, C-type lectin domain family 3 member B, ficolin 3 precursor, glutathione peroxidase precursor, haptoglobin precursor, haptoglobin-related protein precursor, hemopexin precursor, histidine-rich glycoprotein, kallikrein-3, MASP-2-related protein, proapo-A-I protein, prostatic acid phosphatase precursor, relaxin-like factor INSL3, fragment, retinol-binding protein, retinol-binding protein 4, ribonuclease 2, sex hormone-binding globulin, transthyretin precursor, tenascin-X, UBA52, uromodulin, fragment, pigment epithelium-derived factor;  <b>Upregulated proteins:</b> adiponectin precursor, albumin, fragment of, <math>\alpha</math>-1-antitrypsin, <math>\alpha</math>2-HS-glycoprotein precursor (fetuin A), <math>\beta</math>2-microglobulin, <math>\beta</math>-2-glycoprotein 1, calgranulin B, carbonic anhydrase 1, collagen <math>\alpha</math>-1 (II), collagen <math>\alpha</math>-1 (I), collagen <math>\alpha</math>-5 (IV), complement component C4A, complement component C4B3, complement factor H-related 1, complex-forming glycoprotein HC, cubilin, epithelial-cadherin precursor, FAT tumour suppressor, hemopexin, Ig heavy chain, Ig <math>\kappa</math> chain C region, Ig <math>\kappa</math> chain V-II region cum, Ig <math>\kappa</math> chain V-III region SIE, inositol pentakisphosphate 2-kinase, kininogen precursor, megalin, orosomucoid (1-acid glycoprotein), pigment epithelium-derived factor, prostaglandin-H2-isomerase precursor, prostaglandin-H2-isomerase precursor, retinol-binding protein precursor, transthyretin precursor, vitamin D-binding protein, zinc-<math>\alpha</math>2-glycoprotein 1;  <b>Proteins without assessment of up or downregulation:</b> <math>\alpha</math>-1-antitrypsin, <math>\alpha</math>-1-microglobulin, albumin, complement factor B, haptoglobin, hemopexin, orosomucoid, plasma retinol binding, transferrin, transthyretin, zinc <math>\alpha</math>-2-macroglobulin (49, 26).</p>
<p><b>Genes:</b> aldose reductase, vascular endothelial growth factor, angiotensin-I converting enzyme (50); SOD2 (51).</p>
<p><b>The other biomarkers that have been investigated:</b> urinary 8-hydroxydeoxyguanosine (8-OHdG) (52), osteoprotegerin (53), hepatocyte growth factor (HGF) (54,55), matrix metalloproteinase-9 (MMP-9) (56), cystatin C (57), 1,5 anhydroglucitol (58, 59), neutrophil gelatinase-associated lipocalin (NGAL) (60), CA 19-19 (61), HbA1c, fructosamine, glycated albumin (62).</p>

2D-E: Two-dimensional gel electrophoresis; MALDI-MS/MS: Matrix-assisted laser desorption/ionization mass spectrometry/mass spectrometry; SELDI-TOF-MS: Surface enhanced laser desorption/ionization time-of-flight mass spectrometry.

**retin, alpha-1 microglobulin/bikunin precursor, and haptoglobin precursors** as downregulated urinary proteins, and **albumin, zinc alpha-2 glycoprotein, retinal binding proteins** and **E-cadherin** as upregulated urinary proteins in type 2 diabetics, by using proteomic analysis.

The candidate genes involved in the pathways which are dysregulated in diabetes leading to complications have been treated as potential candidate genes for DR. Among approximately 14 genes, only three (**aldose reductase – AKR1B1, vascular endothelial growth factor – VEGF, angiotensin-I converting enzyme – ACE**) were found to be associated with DR (50). The metaanalysis performed by Tian et al. (51) suggested that the C allele of C47T polymorphism in SOD2 gene has protective effects on diabetic microvascular complications, diabetic nephropathy, and diabetic retinopathy.

### Conclusion

As emphasized in this paper, almost all metabolites, products, genes and molecules that are involved in the metabolic abnormalities related to uncontrolled hyperglycemia are candidate biomarkers. The other micro- and macrovascular risk factors for organ damages should also be considered in the assessments. The personalized nature of diabetes and its complica-

tions is another challenging issue, since genetic and environmental factors interact in complex ways. In spite of the findings from the researches and even the recommendations in the guidelines, there is still a gap between the levels of target values of biomarkers to reduce complications and the levels of these targets achieved in actual medical practice. Within the context of these realities, the translational research projects may be helpful for collecting real life data from the managed health care of diabetic patients, besides the bench side researches mentioned above. To accomplish this, the scientific and clinical societies and also the stakeholders in the area of diabetes research and care should work in a collaborative manner in a wide spectrum of disciplines. This may close the gap between the biomarker levels targeted or recommended and the levels achieved in real life, and also provide more relevant biomarkers for the detection of progression and early stages of complications.

In the translational research context, the clinical laboratory may play a significant central role with a properly structured laboratory information system and also data mining tools.

### Conflict of interest statement

The author stated that there are no conflicts of interest regarding the publication of this article.

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