Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes

Aleksandra Klisic1, Nebojsa Kavaric1, Verica Stanisic2, Sanja Vujcic3, Vesna Spasojevic-Kalimanovska3, Ana Ninic3, Jelena Kotur-Stevuljevic1

1Center of Laboratory Diagnostics, Primary Health Care Center, Podgorica, Montenegro
2Department of Radiology, Clinical Center of Montenegro, Podgorica, Montenegro
3Department of Medical Biochemistry, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Submitted: 17 May 2019
Accepted: 12 August 2019

Arch Med Sci 2020; 16 (1): 42–50
DOI: https://doi.org/10.5114/aoms.2019.87541
Copyright © 2019 Termedia & Banach

Abstract

Introduction: We aimed to examine serum endocan level and the summary involvement of dyslipidemia, oxidative stress (OS) and inflammation by calculation of its comprehensive score (i.e. Dyslipidemia-Oxy-Inflammation (DOI) score) in relation to glucoregulation in subjects with prediabetes and overt type 2 diabetes (T2D).

Material and methods: A total of 59 patients with prediabetes and 102 patients with T2D were compared with 117 diabetes-free controls. Glycated hemoglobin (HbA1c), inflammation, OS and lipid parameters were measured. Associations of clinical data with HbA1c level were tested with univariate and multivariate logistic ordinal regression analysis. HbA1c as a dependent variable is given at the ordinal level (i.e. < 5.7%; 5.7–6.4%, > 6.4%, respectively).

Results: Endocan was significantly higher in the T2D group than in the controls. As endocan concentration rose by 1 unit, the probability for higher HbA1c concentration increased by more than 3 times (OR = 3.69, 95% CI: 1.84–7.01, p < 0.001). Also, a rise in the dyslipidemia score, oxy score, inflammation score and DOI score by 1 unit increased the probability of higher HbA1c concentration by 19%, 13%, 51% and 11%, respectively. In the models, after adjustment for confounding variables, endocan and DOI score remained independent predictors of HbA1c level.

Conclusions: Endocan and DOI score are independently correlated with HbA1c in patients with prediabetes and overt T2D.

Key words: inflammation, diabetes, oxidative stress, glycemic control, endocan.

Introduction

The prevalence of type 2 diabetes mellitus (T2D) is increasing worldwide, as are the morbidity and mortality due to its complications [1]. This is mostly related to the modern lifestyle that includes changes in the eating habits, overnutrition, as well as physical inactivity [2].

Hyperglycemia leads to excessive generation of reactive oxygen species (ROS), and when the antioxidant defense capacity is exceeded, it
Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes

Material and methods

Subjects

The current case-control study encompassed a total of 59 patients with prediabetes and 102 with T2D, who were compared with 117 controls. The participants were consecutively recruited when visiting the Primary Health Care Center in Podgorica, Montenegro, for metabolic evaluation by performing laboratory analyses in a period from May 2017 to July 2017.

Each participant filled in a questionnaire consisting of questions regarding demographic characteristics, lifestyle habits (e.g., data about duration of T2D (years), alcohol consumption, cigarette smoking) and somatic illnesses.

The inclusion criteria for participants with prediabetes and T2D were taken from the 2017 American Diabetes Association Standards of Diabetes Care [15]. Participants were considered to have T2D if previously diagnosed T2D or with glycated hemoglobin (HbA1c) level ≥ 6.5% on two different determinations, or with at least two fasting glucose levels ≥ 7.0 mmol/l, or with a random glucose level of ≥ 11.1 mmol/l, or with a glucose level ≥ 11.1 mmol/l, 2 h after an oral glucose tolerance test (OGTT) with 75 g anhydrous glucose dissolved in water.

Participants were considered to have prediabetes if they were not using any antihyperglycemic medications and if they had a fasting glucose ranging between ≥ 5.6 mmol/l and < 7.0 mmol/l, but 2 h after OGTT had a plasma glucose level between 7.8 mmol/l and 11.1 mmol/l, or if they exhibited a HbA1c level between 5.7% and 6.4%.

The control group consisted of diabetes-free participants who were not using any antihyperglycemic medications, with a HbA1c level lower than 5.7% and fasting glucose lower than 5.6 mmol/l.

Participants with severe anemia, a history of acute myocardial infarction or stroke in the last 6 months, type 1 diabetes mellitus, high-sensitivity C-reactive protein (hsCRP) > 10 mg/l, thyroid dysfunction, hepatic disease other than steatosis, renal disease other than diabetic nephropathy and with estimated glomerular filtration rate (eGFR) < 15 ml/min/1.73 m², patients with ethanol consumption > 20 g/day and pregnancy were excluded from the study.

Each participant signed consent and was well informed about the aim of the study. The Ethics Committee of the Primary Health Care Center in Podgorica, Montenegro approved the protocol of the study.

A total of 86% of T2D participants used oral antihyperglycemics (of them metformin, sulfonylureas, and inhibitors of dipeptidyl peptidase 4 (DPP-4 inhibitors) were used by 94%, 8%, and 10%
of patients, respectively), whereas 30% of them were on insulin therapy. There were no participants in the control and prediabetes group who used antihyperglycemic medications, as would be expected based on inclusion/exclusion criteria. Hypolipidemics were used by 24%, 29% and 47% of participants in the control, prediabetes and T2D group, respectively. All of them used statins in the control and prediabetes group, whereas 96% of participants in the T2D group used statins and 4% of them used fibrates. Antihypertensive drugs were used by 46%, 63% and 83% of participants in the control, prediabetes and T2D group, respectively.

Anthropometric measurements were taken from each participant (i.e., body height (cm), body weight (kg), and waist circumference (WC), whereas body mass index (BMI) was calculated).

Systolic (SBP) and diastolic blood pressure (DBP) were measured with a mercury sphygmomanometer after the participant’s rest for 5 minutes. All measurements were taken by the same nurse and recorded the average of the 3 measurements taken on the right arm.

The estimation of glomerular filtration rate (eGFR\textsubscript{MDRD}) was calculated by using Modification of Diet in Renal Disease Study equation, as described previously [4].

Methods

The phlebotomy was performed and blood samples were taken in the morning after an overnight fast of at least 8 h. Samples collected in tubes with serum separator and clot activator were left to clot for 30 min, and thereafter were centrifuged at 3000xg. Afterwards, serum samples were divided into aliquots and stored at −80°C for determination of endocan, high-sensitivity C-reactive protein (hsCRP) and OS markers, whereas the remaining sera were analyzed immediately for fasting glucose, lipid parameters (i.e., triglycerides, TG, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and creatinine. Samples of whole blood in tubes containing K\textsubscript{2}EDTA were used for determination of endocan, high-sensitivity C-reactive protein (hsCRP) and OS markers.

Serum hsCRP levels were measured by nephelometric assay (Behring Nephelometer Analyzer, Marburg, Germany). Endocan levels were measured using an enzyme-linked immunosorbent commercial assay (ab213776 – Human ESM1 ELISA Kit, Abcam, Cambridge, UK). Ninety-six well plates were coated with mouse monoclonal antibody specific for ESM1/endocan with samples added afterwards. A biotinylated detection polyclonal antibody from goat specific for ESM1/endocan was added subsequently and then rinsed three times in PBS or TBS buffer. Avidin-biotin-peroxidase complex was added (PBS or TBS buffer washed away unbound conjugates). HRP substrate TMB was added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP resulting with the blue colored product. After adding TMB stop solution the blue product becomes yellow which is proportional to the concentration of human ESM1/endocan. The absorbance was read at 450 nm in a microplate reader for 30 min.

Determination of prooxidant-antioxidant balance (PAB) was performed using 3,3′, 5,5′-tetramethylbenzidine as a chromogen [16]. Advanced oxidation protein products (AOPP) were measured by a method that used a reaction with glacial acetic acid and potassium iodide [17]. Total oxidative status (TOS) was determined spectrophotometrically using o-dianisidine optimized by Erel [18]. Total antioxidative status (TAS) was evaluated spectrophotometrically using ABTS as a chromogen [19]. Total protein sulfhydryl (SH-) groups were measured spectrophotometrically using 5,5′-dithiobis (2-nitrobenzoic acid) [20].

Calculation of novel scores

Since we aimed to explore the summary effect of the key risk factors in patients with prediabetes and T2D (i.e. dyslipidemia, OS and inflammation), we calculated the DOI score as the sum of the dyslipidemia score, oxy score and inflammation score [21, 22].

The dyslipidemia score was calculated after standardization of all the variables and calculating z scores for HDL-C, LDL-C, and TG and using mean and standard deviation calculated from the healthy control group. Afterwards, standardized HDL-C was subtracted from the average of the standardized variables (TG + LDL-C) [21, 22].

The oxy score was obtained by subtraction of the protective score (i.e. which was calculated as an average of standardized antioxidant variables (total SH- groups and TAS)) from the damage score (i.e. which was calculated as the average of standardized prooxidant factors (AOPP, TOS, and PAB)) [23].

The inflammation score was determined after standardization of hsCRP [21].

Statistical analysis

Before the start of the study, it was calculated that to achieve a power of 80% and a level of significance of 5% (two-sided) for detecting a true difference in means between the test and the reference group, a total of 111 participants in each of the control and T2D group was required. We
Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes.

Included 10% more participants, but due to exclusion criteria, finally a total of 117 participants were included in the control group and 102 participants in the T2D group. However, we could not perform a sample size determination for the group with prediabetes because there were no previous studies that examined endocan levels in patients with prediabetes.

After testing data distribution by Shapiro-Wilk test, log-normally distributed data were presented as the geometric mean (95% confidence interval – 95% CI) and skewed distributed data as the median (interquartile range). Comparison of log-normal data was performed by one-way ANOVA with the Tukey post hoc test and comparison of skewed data was performed by the Kruskal-Wallis test when testing differences between three groups, and the Mann-Whitney test when testing the difference between two groups. Categorical data were presented as absolute and relative frequencies and compared by the χ² test for contingency tables. Spearman’s bivariate correlation analysis was used for testing the associations between clinical parameters, and the data were presented as correlation coefficients (ρ). In-depth independent associations of clinical data with HbA1c level were tested with univariate and multivariate logistic ordinal regression analysis. Independent variables were given as continuous or dichotomous with no multicollinearity among them and each had an identical effect at each cumulative split of the ordinal dependent variable. Also, continuous independent variables included in models were correlated with HbA1c. HbA1c, as a dependent variable is given at the ordinal level (1 for a HbA1c

| Parameter | Control group | Prediabetes | T2D | P-value |
|-----------|---------------|-------------|-----|---------|
| N (male)  | 117 (30%)     | 59 (34%)    | 102 (53%) | 0.001 |
| Age [years] | 58 (56–60)   | 62 (60–65)** | 65 (63-67)** | < 0.001 |
| BMI [kg/m²] | 27.3 (26.6–28.1) | 28.5 (27.4–29.6) | 30.3 (29.5–31.2)** | < 0.001 |
| WC [cm]† | 95 (87–102) † | 96 (91–102) † | 104 (97–110)** † | < 0.001 |
| SBP [mm Hg] † | 136 (124–146) | 136 (126–150) | 133 (126–148) | 0.642 |
| DBP [mm Hg] † | 84 (77–91) † | 85 (76–92) † | 84 (77–94) † | 0.921 |
| Smokers, n (%) | 25 (21) | 19 (32) | 14 (14) | 0.021 |
| Antihyperglycemics, n (%): | – | – | 88 (86) | – |
| Metformin | 83 (94) | – | – | – |
| Sulfonylurea | 7 (8) | – | – | – |
| DPP-4 inhibitors | 9 (10) | – | – | – |
| Insulin, n (%) | – | – | 31 (30) | – |
| Hypolipidemics, n (%): | 28 (24) | 17 (29) | 48 (47) | 0.001 |
| Statins | 28 (100) | 17 (100) | 46 (96) | – |
| Fibrates | – | – | 2 (4) | – |
| Antihypertensives, n (%): | 54 (46) | 37 (63) | 85 (83) | < 0.001 |
| ACE | 47 (87) | 30 (81) | 67 (79) | – |
| CCB | 3 (5) | 1 (3) | 8 (9) | – |
| ARB | 3 (5) | 1 (3) | 8 (9) | – |
| Beta-blockers | 15 (32) | 7 (19) | 27 (32) | – |
| Thiazide diuretics | 26 (48) | 18 (49) | 49 (58) | – |
| Duration of diabetes, years | – | – | 5 (1–10) | – |

Data are presented as geometric mean (95% CI) and compared by one-way ANOVA with Tukey post hoc test. †Data are presented as median (interquartile range) and compared by Kruskal-Wallis or Mann-Whitney test dependent on the number of examined groups. *P for one way ANOVA or Kruskal-Wallis test. **Significantly different from the control group; ***significantly different from the prediabetes group. *P < 0.05; **P < 0.01. BMI – body mass index, WC – waist circumference, SBP – systolic blood pressure, DBP – diastolic blood pressure, DPP-4 inhibitors – inhibitors of dipeptidyl peptidase 4, ACE inhibitors – angiotensin converting enzyme inhibitors, CCB – calcium channel blocker, ARB – angiotensin receptor blocker.
concentration less than 5.7%; 2 for a HbA1c level between 5.7 and 6.4%; and 3 for a HbA1c concentration higher than 6.4% or lower only if the patient self-reported T2D). Data from logistic ordinal regression analyses were presented as odds ratio (OR) and 95% CI. The explained variation in HbA1c level in the tested population was given by the Nagelkerke $R^2$ value.

All statistical testing was performed using the statistical program IBM SPSS Statistics version 22 (SPSS Inc., Chicago, Illinois, USA). The statistical significance level was set at two-sided $p < 0.05$.

**Results**

There were unequal numbers of women and men were in the tested groups. There were unequal numbers of women and men in the tested groups. The youngest examinees were those in the control group. Patients with T2D had the highest BMI and WC compared to controls and prediabetes patients. Smokers were most prevalent in the prediabetes group. Patients on hypolipidemic and antihypertensive therapies were more frequent among patients with T2D (Table I).

Most of the tested clinical markers, except for TAS, TOS and total SH groups, were significantly different between tested groups (Table II). There were significant differences in glucose and HbA1c level between all three groups, being the highest in the T2D and the lowest in the control group. The lowest TC, LDL-C and PAB levels were in the group with T2D. HDL-C level was the highest in the control group. Creatinine concentration was the highest in the T2D group. Patients with prediabetes and T2D had significantly higher hsCRP and AOPP levels than the control group. TG and endocan levels were significantly different only between the control group and T2D, being higher in the group of patients with T2D.

The Kruskal-Wallis test showed significant differences in all calculated scores and all of them were significantly lower in the control group compared to the prediabetes and T2D groups (Table III). The scores showed no difference between the prediabetes and T2D groups.

Results from Spearman’s correlation analysis between HbA1c and other variables are presented in Table IV. Significant positive correlations were established between HbA1c and the following variables: years of age, BMI, WC, glucose, TG, creatinine, hsCRP, AOPP, TAS, total SH groups and calculated scores in all participants. Significant negative correlations were observed between

### Table II. Clinical markers of tested population groups

| Parameter | Control group | Prediabetes | T2D | P-value* |
|-----------|---------------|-------------|-----|----------|
| Glucose [mmol/l] | 5.4 (5.2–5.6) | 6.1 (5.8–6.5) | 7.9 (6.7–9.6) | < 0.001 |
| HbA1c (%) | 5.3 (5.1–5.5) | 5.9 (5.7–6.0) | 7.1 (6.5–8.9) | < 0.001 |
| Total cholesterol [mmol/l] | 5.80 (5.57–6.04) | 6.02 (5.64–6.43) | 5.23 (5.02–5.46) | < 0.001 |
| HDL cholesterol [mmol/l] | 1.51 (1.44–1.58) | 1.31 (1.20–1.44) | 1.20 (1.14–1.26) | < 0.001 |
| LDL cholesterol [mmol/l] | 3.42 (3.23–3.63) | 3.73 (3.42–4.07) | 2.92 (2.72–3.13) | < 0.001 |
| TG [mmol/l] | 1.54 (1.42–1.67) | 1.77 (1.56–2.00) | 2.06 (1.88–2.26) | < 0.001 |
| Creatinine [μmol/l] | 70 (62–84) | 72 (66–71) | 80 (67–93) | < 0.001 |
| eGFR MDRD [mL/min/1.73 m²] | 82 (73–91) | 77 (70–86) | 73 (62–86) | 0.001 |
| HsCRP [mg/l] | 0.88 (0.75–1.04) | 1.31 (1.02–1.68) | 1.54 (1.29–1.84) | < 0.001 |
| AOPP [μmol/l] | 38.84 (36.89–40.89) | 45.82 (41.57–50.49) | 47.12 (44.14–50.31) | < 0.001 |
| PAB [HKU] | 103 (96–110) | 104 (93–115) | 88 (81–95) | 0.005 |
| TOS [μmol/l] | 9.09 (7.81–10.58) | 10.30 (8.22–12.90) | 11.09 (9.63–12.76) | 0.180 |
| TAS [μmol/l] | 1130 (1077–1186) | 1120 (1075–1166) | 1162 (1108–1220) | 0.562 |
| Total SH- groups [μmol/l] | 0.237 (0.219–0.256) | 0.270 (0.248–0.295) | 0.269 (0.248–0.293) | 0.065 |
| Endocan [ng/ml] | 0.282 (0.246–0.323) | 0.308 (0.248–0.383) | 0.395 (0.330–0.473) | 0.002 |

Data are presented as geometric mean (95% CI) and compared by one-way ANOVA with Tukey post hoc test. *Data are presented as median (interquartile range) and compared by Kruskal-Wallis test or Mann-Whitney test dependent on the number of examined groups. *P for one-way ANOVA or Kruskal-Wallis test. **Significantly different from the control group. ***Significantly different from the prediabetes group. $^*$P < 0.05; **P < 0.01. HbA1c – glycated hemoglobin, HDL cholesterol – high-density lipoprotein cholesterol, LDL cholesterol – low-density lipoprotein cholesterol, TG – triglycerides, eGFR MDRD – estimated glomerular filtration rate, HsCRP – high-sensitivity C-reactive protein, AOPP – advanced oxidation protein products, PAB – prooxidant-antioxidant balance, TOS – total oxidative status, TAS – total antioxidative status, Total SH- groups – total protein sulfhydryl (SH-) groups.
Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes.

Table III. Dyslipidemia score, oxy score, inflammation score and DOI Score in examined groups

| Parameter         | Controls          | Prediabetes       | T2D              | P-value |
|-------------------|-------------------|-------------------|------------------|---------|
| Dyslipidemia score| –0.72 (–1.89)–(0.14) | –0.19 (–1.10)–(1.41)* | 0.23 (–1.04)–(1.14) ** | 0.003 |
| Inflammation score| –0.59 (–0.78)–(0.26) | –0.28 (–0.71)–0.40* | –0.31 (–0.67)–0.27** | 0.002 |
| Oxy score         | 4.38 (2.74–6.70)  | 6.57 (4.16–9.11)*  | 6.65 (4.63–10.26)* | < 0.001 |
| DOI score         | 3.55 (0.68–6.14)  | 7.41 (2.62–10.50)* | 6.99 (2.70–11.92)* | < 0.001 |

Data are given as median (interquartile range) and compared by Kruskal-Wallis test or Mann-Whitney test dependent on the number of examined groups. *Significantly different from the control group. *P < 0.05; **p < 0.01.

HbA1c, and total cholesterol, HDL-C, LDL-C and PAB in all participants.

Further we wanted to test whether endocan and scores were independently associated with HbA1c concentration using logistic ordinal regression analysis (Table V). Significant ORs obtained in univariate analysis were evident for all tested parameters. This analysis indicated that a rise in endocan concentration by 1 unit increased the probability of higher HbA1c concentration 3 times (OR = 3.69, 95% CI: 1.84–7.01, p < 0.001). Also, a rise in dyslipidemia score, oxy score, inflammation score and DOI score by 1 unit increased the probability of higher HbA1c concentration by 19%, 13%, 51% and 11%, respectively. When tested in different models, endocan, dyslipidemia score and DOI score were found to be independent predictors of HbA1c level. In all four models (Models 1–4), as endocan concentration rose by 1 unit, the probability of a higher HbA1c level increased by 2.66, 2.31, 2.64 and 2.28 times, respectively. As dyslipidemia score (Model 1) and DOI score (Model 4) rose by 1 unit, the probability of a higher HbA1c level increased by 31% and 9%, respectively. Nagelkerke $R^2$ for Models 1–4 were 0.366, 0.395, 0.395 and 0.359, respectively, which means that 36.6%, 39.5%, 39.5% and 35.9% of variation in HbA1c level could be explained by parameters included in the models.

Discussion

To the best of our knowledge, this is the first study that has estimated serum endocan levels and the summary involvement of dyslipidemia, OS and inflammation by comprehensive score calculation (i.e., DOI score) in relation to glucose regulation in patients with prediabetes and T2D. Although previous studies investigated separately some of the biomarkers that were used for calculation of these scores in patients with T2D, we presumed that the mutual effect of several of these biomarkers calculated as DOI score could better explain the complex interrelationship between glucose regulation and these features, rather than examining each single biomarker. Additionally, endocan is not explored thoroughly in different stages of diabetes, such as prediabetes and overt T2D.

Table IV. Spearman’s correlation analysis between HbA1c and clinical markers in all participants

| Parameter | Rho ($\rho$) | P-value |
|-----------|--------------|---------|
| Age [years] | 0.311** |         |
| BMI [kg/m²] | 0.282** |         |
| WC [cm] | 0.351** |         |
| SBP [mm Hg] | 0.002 |         |
| Glucose [mmol/l] | 0.744** |         |
| Total cholesterol [mmol/l] | –0.165* |         |
| HDL cholesterol [mmol/l] | –0.334** |         |
| LDL cholesterol [mmol/l] | –0.157** |         |
| TG [mmol/l] | 0.254** |         |
| Creatinine [μmol/l] | 0.207** |         |
| HsCRP [mg/l] | 0.239** |         |
| ADPP [μmol/l] | 0.284** |         |
| PAB [HKU] | –0.143* |         |
| TOS [μmol/l] | 0.130* |         |
| Total SH- groups [μmol/l] | 0.180** |         |
| Endocan [ng/ml] | 0.157** |         |
| Dyslipidemia score | 0.169** |         |
| Inflammation score | 0.165** |         |
| Oxy-score | 0.299** |         |
| DOI score | 0.239** |         |

*P < 0.05, **p < 0.01, HbA1c – glycated hemoglobin, BMI – body mass index, WC – waist circumference, SBP – systolic blood pressure, DBP – diastolic blood pressure, HDL cholesterol – high-density lipoprotein cholesterol, LDL cholesterol – low-density lipoprotein cholesterol, TG – triglycerides, HsCRP – high-sensitivity C-reactive protein, ADPP – advanced oxidation protein products, PAB – prooxidant-antioxidant balance, TOS – total oxidative status, TAS – total antioxidative status, Total SH- groups – total protein sulfhydryl (SH-) groups.

In line with this, we have shown that serum endocan levels were significantly higher in T2D group than in the controls. Although median values of endocan levels were higher in the predia-
Table V. Estimated odds ratios after ordinal regression analysis for HbA\textsubscript{1c} cut-offs as dependent variable

| Parameter                  | Unadjusted OR (95% CI) | P-value | Nagelkerke R\textsuperscript{2} |
|----------------------------|------------------------|---------|-------------------------------|
| Endocan [ng/ml]            | 3.69 (1.84–7.01)       | < 0.001 | 0.076                         |
| Dyslipidemia score         | 1.19 (1.06–1.34)       | 0.004   | 0.034                         |
| Oxy-score                  | 1.13 (1.07–1.20)       | < 0.001 | 0.083                         |
| Inflammation score         | 1.51 (1.16–1.96)       | 0.002   | 0.042                         |
| DOI score                  | 1.11 (1.06–1.16)       | < 0.001 | 0.102                         |

Model 1 Adjusted

| Parameter                  | OR (95% CI) | P-value | Nagelkerke R\textsuperscript{2} |
|----------------------------|-------------|---------|-------------------------------|
| Endocan [ng/ml]            | 2.66 (1.32–5.37) | 0.006   | 0.366                         |
| Dyslipidemia score         | 1.31 (1.12–1.53) | 0.001   |                               |

Model 2 Adjusted

| Parameter                  | OR (95% CI) | P-value | Nagelkerke R\textsuperscript{2} |
|----------------------------|-------------|---------|-------------------------------|
| Endocan [ng/ml]            | 2.31 (1.03–5.16) | 0.042   | 0.395                         |
| Oxy score                  | 0.98 (0.89–1.07) | 0.628   |                               |

Model 3 Adjusted

| Parameter                  | OR (95% CI) | P-value | Nagelkerke R\textsuperscript{2} |
|----------------------------|-------------|---------|-------------------------------|
| Endocan [ng/ml]            | 2.64 (1.29–5.39) | 0.008   | 0.395                         |
| Inflammation score         | 1.17 (0.21–2.17) | 0.230   |                               |

Model 4 Adjusted

| Parameter                  | OR (95% CI) | P-value | Nagelkerke R\textsuperscript{2} |
|----------------------------|-------------|---------|-------------------------------|
| Endocan [ng/ml]            | 2.28 (1.02–5.10) | 0.043   | 0.359                         |
| DOI score                  | 1.09 (1.03–1.15) | 0.001   |                               |

Data are given as OR (95% CI). Model 1 included continuous variables: age, WC, creatinine, hsCRP, endocan, dyslipidemia score and categorical variables: gender, smoking habits, antihypertensive therapy, hypolipidemic therapy. Model 2 included continuous variables: age, WC, creatinine, HDL-c, TG, hsCRP, endocan, oxy score and categorical variables: gender, smoking habits, antihypertensive therapy, hypolipidemic therapy. Model 3 included continuous variables: age, WC, creatinine, HDL-c, TG, endocan, inflammation score and categorical variables: gender, smoking habits, antihypertensive therapy, hypolipidemic therapy. Model 4 included continuous variables: age, WC, creatinine, endocan, DOI score and categorical variables: gender, smoking habits, antihypertensive therapy, hypolipidemic therapy.

In the study of Balamir et al. [25], serum endocan levels were shown to be associated with endothelial dysfunction in T2D patients. Also, a recent study of Lv et al. [26] suggested that serum endocan might be a reliable parameter for

Inflammation is one of the underlying risks of β cell damage and IR in patients with T2D [2]. Furthermore, the vicious circle of hyperinsulinemia and hyperglycemia and the prolonged burden of pro-inflammatory cytokines lead to deeper imbalance in the lipoprotein levels, promote lipid peroxidation, as well as oxidative modification of protein structures, and aggravate endothelial dysfunction and atherosclerosis [6, 24]. Having these facts in mind, a novel inflammation biomarker of endothelial dysfunction, such as endocan [3], and a comprehensive DOI score which includes all these key features of T2D may be important determinants of the progression of this metabolic disorder.

The current study has also for the first time shown that all examined novel scores (i.e., oxy-score, inflammation, dyslipidemia and its summary DOI score) were significantly higher in the prediabetes and T2D group, as compared with the control group. Furthermore, our research showed that endocan and summary DOI score are independently correlated with HbA\textsubscript{1c} level in examined population groups.
Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes.

In conclusion, the current study has for the first time shown that endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with HbA1c in patients with prediabetes and T2D. Knowing that poor glucose regulation represents an important risk factor for CVD, future studies are needed to examine whether determination of these biomarkers in a timely manner may be useful to detect atherosclerotic disease risk burden in (pre)diabetes, and to investigate whether lowering it can enable prevention of T2D and/or its cardiometabolic complications.

Acknowledgments
This work was financially supported in part by a grant from the Ministry of Science, Montenegro and Ministry of Education, Science and Technological Development, Republic of Serbia (project number 175035).

Conflict of interest
The authors declare no conflict of interest.

References
1. Yaribeygi H, Atkin SL, Sahebkar A. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. J Cell Physiol 2019; 234: 1300-12.
2. Papaetis GS, Papakyriakou R, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci 2015; 11: 463-82.
3. Balta S, Mikhailidis DP, Demirkol S, Ozturk C, Celik T, Isik C. Endocan: a novel inflammatory indicator in cardiovascular disease? Atherosclerosis 2015; 243: 339-43.
4. Kilaric A, Kavicar N, Jovanovic M, et al. Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci 2017; 22: 122.
5. Kavicar N, Klisic A, Ninic A. Are Visceral Adiposity Inflammation (VAPI) Index and Lipid Accumulation Product reliable indices for metabolic disturbances in patients with type 2 diabetes mellitus? J Clin Lab Anal 2018; 32: e22283.
6. Ungurianu A, Margina D, Grădianu D, et al. Lipoprotein redox status evaluation as a marker of cardiovascular disease risk in patients with inflammatory disease. Mol Med Rep 2016; 15: 256-62.
7. Zhao T, Kecheng Y, Zhao X, et al. The higher serum endocan levels may be a risk factor for the onset of cardiovascular disease: a meta-analysis. Medicine (Baltimore) 2018; 97: e13407.
8. Qiu CR, Fu Q, Sui J, et al. Analysis of serum endothelial cell-specific molecule 1 (endocan) level in type 2 diabetes mellitus with acute ST-segment elevation myocardial infarction and its correlation—a pilot study. Angiology 2017; 68: 74-8.
9. Lee W, Ku SK, Kim SW, Bae JS. Endocan elicits severe vascular inflammatory responses in vitro and in vivo. J Cell Physiol 2014; 229: 620-30.
10. Ekiz-Bilir B, Bilir B, Aydin M, Soysal-Atile N. Evaluation of endocan and endoglin levels in chronic kidney disease due to diabetes mellitus. Arch Med Sci 2019; 15: 86-91.
11. Arman Y, Akpinar TS, Kose M, et al. Effect of glycemic regulation on endocan levels in patients with diabetes: a preliminary study. Angiology 2016; 67: 239-44.
12. Rodrigues KF, Pietrani NT, Bosco AA, et al. Endocan: a new biomarker associated with inflammation in type 2 diabetes mellitus? Diabetes Metab Res Rev 2015; 31: 479-80.
13. Kacic A, Isaiovic A, Kocic G, et al. Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2018; 126: 371-8.
14. Kacic A, Kocic G, Kavaric N, Jovanovic M, Stanisic V, Ninic A. Xanthine oxidase and uric acid as independent predictors of albuminuria in patients with diabetes mellitus type 2. Clin Exp Med 2018; 18: 283-90.
15. American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes-2017. Diabetes Care 2017; 40: S11-24.
16. Alamdari DH, Paletas K, Pegiou T, Sarigianni M, Befani C, Koliakos G. A novel assay for the evaluation of the pro-oxidant-antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients. Clin Biochem 2007; 40: 248-54.
17. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49: 1304-13.
18. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-11.
19. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37: 277-85.
20. Elmajian D. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-7.
21. Blagojević IP, Ignjatović S, Macut D, et al. Evaluation of a summary score for dyslipidemia, oxidative stress and inflammation (the Doi Score) in women with polycystic ovary syndrome and its relationship with obesity. J Med Biochem 2018; 37: 477-85.
22. Wrzosek M, Sawicka A, Wrzosek M, Piątkiewicz P, Talataj M, Nowicka G. Age at onset of obesity, transcription factor 7-like 2 (TCF7L2) rs7903146 polymorphism, adiponectin levels and the risk of type 2 diabetes in obese patients. Arch Med Sci 2019; 15: 321-9.
23. Veglia F, Cighetti G, De Franceschi M, et al. Age- and gender-related oxidative status determined in healthy subjects by means of OXY-SCORE, a potential new comprehensive index. Biomarkers 2006; 11: 562-73.
24. Atwa H, Gad K, Hagrasy H, et al. Is subclinical atherosclerosis associated with visceral fat and fatty liver in adolescents with type 1 diabetes? Arch Med Sci 2018; 14: 1335-60.
25. Balamir I, Ates I, Topcuoglu C, Turhan T. Association of endocan, ischemia-modified albumin, and hsCRP levels with endothelial dysfunction in type 2 diabetes mellitus. Angiology 2018; 69: 609-16.
26. Lv Y, Zhang Y, Shi W, et al. The association between endocan levels and subclinical atherosclerosis in patients with type 2 diabetes mellitus. Am J Med Sci 2017; 353: 433-8.
27. Parizadeh SM, Azarpazhooh MR, Mohebati M, et al. Simvastatin therapy reduces prooxidant-antioxidant balance: results of a placebo-controlled cross-over trial. Lipids 2011; 46: 333-40.
28. Celik T, Balta S, Karaman M, et al. Endocan, a novel marker of endothelial dysfunction in patients with essential hypertension: comparative effects of amlodipine and valsartan. Blood Press 2015; 24: 55-60.
29. Caballero AE, Delgado A, Aguilarr-Salinas CA, et al. The differential effects of metformin on markers of endothelial activation and inflammation in subjects with impaired glucose tolerance: a placebo-controlled, randomized clinical trial. J Clin Endocrinol Metab 2004; 89: 3943-8.
30. Tziomalos K, Athyros VG, Karagiannis A, Mikhalidis DP. Lipid lowering agents and the endothelium: an update after 4 years. Curr Vasc Pharmacol 2012; 10: 33-41.
31. Wrzosek M, Sawicka A, Wrzosek M, Piątkiewicz P, Talataj M, Nowicka G. Age at onset of obesity, transcription factor 7-like 2 (TCF7L2) rs7903146 polymorphism, adiponectin levels and the risk of type 2 diabetes in obese patients. Arch Med Sci 2019; 15: 321-9.
32. Totomoch-Serra A, Muñoz M, Burguero J, Revilla-Monsalve M, Díaz-Badillo A. Association of common polymorphisms in the VEGFA and SIRT1 genes with type 2 diabetes-related traits in Mexicans. Arch Med Sci 2018; 14: 1361-73.