https://doi.org/10.34883/PI.2024.16.3.008

Manwar I.Q. ✉, Kata F.S.
University of Basrah, Basrah, Iraq

# Physiological Evaluation of Endothelin-1 and Osteoprotegerin as a Risk Predicator for Cardiovascular Diseases in Diabetic Patients

**Conflict of interest:** nothing to declare.
**Authors' contribution:** Manwar I.Q. – conceptualization, methodology, formal analysis, investigation, resources, data curation, writing – original draft; Kata F.S. – supervision, investigation, resources, data curation, writing – original draft.
The article is published in the author's edition.

Submitted: 17.01.2024
Accepted: 08.04.2024
Contacts: medicalresearch55@yahoo.com

**Abstract**

**Purpose.** To estimate the levels of the biomarkers Endothelin-1 (ET-1) and Osteoprotegerin (OPG) in the serum of diabetes mellitus women as biomarkers for predicting cardiovascular disease.
**Materials and methods.** A total of 88 samples from women, 60 samples healthy women, and 28 patients with diabetes type 2. As the samples were divided according to the severity of the disease based on HbA1c, 32 serum samples for women whose HbA1c ranged between 7–9.9, and 28 serum samples for women whose HbA1c ranged between 10–14. Also, the samples were divided according to the time period of the disease, 36 serum samples for women with a period of illness ranging from 5–10 years and 24 serum samples for women with a period of illness ranging from 11–22 and the samples were divided according to the age of patients, 29 serum samples for women with an age ranging from 45–54 years, and 31 serum samples for women with an age ranging from 55–70.
**Results.** There is a significant increase level ET-1 at P≤0.01 in the diabetes group compared with the healthy. There was also a significant increase in the concentration biomarker OPG in the Diabetes group compared with the healthy.
**Conclusion.** The significant rising in the level of biomarkers may be one of the induce reasons of cardiovascular diseases in patients with diabetes, therefore its adoption as risk indicators for early prediction of the occurrence of cardiovascular diseases.
**Keywords:** diabetes mellitus, endothelin-1, osteoprotegerin, cardiovascular disease, biomarkers

Манвар И.К. ✉, Ката Ф.С.
Университет Басры, Басра, Ирак

# Физиологическая оценка эндотелина-1 и остеопротегерина как предикторов риска развития сердечно-сосудистых заболеваний у пациентов с сахарным диабетом

**Конфликт интересов:** не заявлен.
**Вклад авторов:** Манвар И.К. – концепция исследования, методология, анализ данных, проведение исследования, ресурсы, обработка данных, написание чернового варианта статьи; Ката Ф.С. – научное руководство, проведение исследования, ресурсы, обработка данных, написание чернового варианта статьи.
Статья опубликована в авторской редакции.

Подана: 17.01.2024
Принята: 08.04.2024
Контакты: medicalresearch55@yahoo.com

**Резюме**

**Цель**. Оценка концентрации биомаркеров эндотелина-1 (ЭТ-1) и остеопротегерина (ОПГ) как биомаркеров для прогнозирования сердечно-сосудистых заболеваний в сыворотке крови женщин с сахарным диабетом.
**Материалы и методы.** Всего было отобрано 88 образцов, из них 60 образцов здоровых женщин и 28 – пациенток с диабетом 2-го типа. Образцы распределяли (классифицировали) следующим образом: по тяжести заболевания на основании теста на HbA1c (32 образца сыворотки женщин с показателем HbA1c 7–9,9 и 28 образцов сыворотки женщин с показателем HbA1c 10–14), по продолжительности заболевания (36 образцов сыворотки женщин с продолжительностью заболевания 5–10 лет и 24 образца сыворотки женщин с продолжительностью заболевания 11–22 года), а также по возрасту испытуемых (29 образцов сыворотки женщин в возрасте 45–54 лет и 31 образец сыворотки женщин в возрасте 55–70 лет).
**Результаты.** Выявлено достоверное повышение уровня ЕТ-1 при $P \leq 0{,}01$ в группе пациентов с диабетом по сравнению с группой здоровых испытуемых. Также отмечено достоверное увеличение концентрации биомаркера ОПГ в группе пациентов с диабетом по сравнению с группой здоровых испытуемых.
**Заключение.** Достоверное повышение уровня биомаркеров может свидетельствовать о развитии сердечно-сосудистых заболеваний у пациентов с сахарным диабетом, что позволяет рассматривать их в качестве индикаторов при прогнозировании сердечно-сосудистого риска на ранней стадии.
**Ключевые слова:** сахарный диабет, эндотелин-1, остеопротегерин, сердечно-сосудистые заболевания, биомаркеры

## ■ INTRODUCTION

Diabetes is associated with a group of metabolic disorders that result in elevated blood sugar levels, hyperglycemia, beyond the normal ranges, chronic hyperglycemia in diabetes is linked to long-term damage and dysfunction of various organs, especially the nerves, heart, and blood vessels [1].

It is well known that the prominent features of diabetes complications include arterial stiffness, endothelial dysfunction, low-grade inflammation of the heart, impaired tissue healing due to insulin resistance, elevated blood sugar levels, and oxidative stress [2]. All these factors contribute to the development of coronary artery disease, diabetic cardiomyopathy, heart failure, arrhythmias, and the risk of sudden death [3]. Biological indicators (Biomarkers) can predict the appropriate diagnosis of certain diseases and predict heart disease mortality, as well there is a clear association between diabetes and cardiovascular diseases, with diabetes itself being a risk factor for cardiovascular and vascular complications such as myocardial infarction, stroke, arterial stiffness, and hypertension [4, 5].

Biomarkers are considered as tools for predicting certain physiological conditions, and they are used for the early detection and assessment of certain diseases such as diabetes, hyperlipidemia, metabolic syndrome, hypertension, and cardiovascular diseases [6, 7].

Endothelin-1 (ET-1) is one of these biomarkers and It is derived from vascular endothelial cells and composed of 21 amino acids and it has a potent and sustained vasoconstrictive effect [8]. Zhang et al. [9] indicated that ET-1 levels increase in patients with arterial stiffness, and its level can reflect the severity of vascular damage. Similarly, a study by Chen et al. [10] found that ET-1 levels were elevated in patients with cardiovascular diseases compared to healthy individuals. In another study of a group of patients undergoing coronary artery bypass graft surgery (CABG), it was observed that ET-1 levels were higher in diabetic patients compared to non-diabetic individuals [11]. ET-1 mediates pathological processes such as inflammation, oxidative stress, endothelial dysfunction, and insulin resistance, leading to the development and progression of diabetes and arterial sclerosis [12].

Regarding osteoprotegerin (OPG), it is a soluble protein belonging to the tumor necrosis factor receptor superfamily, and it is present freely in the blood and not bound to the cell membrane [13]. OPG is among the many molecules that have been studied for their potential capability as biomarkers of cardiovascular diseases (CVD) in diabetic patients, with increased attention given to this family of tumor necrosis factor receptors (TNF) [14].

OPG levels have been shown to serve as an independent biomarker for cardiovascular and CVD in patients with acute or chronic heart diseases. Additionally, OPG has been implicated in various inflammations and the development of diabetes [15]. It has been observed that OPG levels increase in parallel with the severity of diabetic complications. The higher levels of osteoprotegerin have been noticed in diabetic patients who also suffer from heart and cardiovascular diseases, Although the clinical use of OPG may seem distant, it appears promising as a biological marker However, OPG shows promise as a biomarker to aid cardiologists better classify disease risk in in diabetic patients Recently, the attention has shifted towards the relationship between bone-regulating proteins and vascular biology, suggesting that OPG may act as a potential mediator of vascular calcification [16]. It also plays a role in stimulating inflammation, vascular injury, and atherosclerosis [14]. Furthermore, there is an association between OPG levels in serum and the severity and prediction of acute coronary syndrome [17].

Despite the fact that physiological levels of OPG primarily prevent arterial calcification, it has been observed that elevated plasma levels significantly contribute to vascular dysfunction and inflammation. It has been proposed that increased levels of OPG in the blood serve as a vital indicator of arterial stiffness and cardiovascular diseases [18, 19].

## ■ PURPOSE OF THE STUDY

To estimate the levels of the biomarkers Endothelin-1 (ET-1) and Osteoprotegerin (OPG) in the serum of diabetes mellitus women as a biomarkers for predicting cardiovascular disease.

## ■ MATERIALS AND METHODS

Samples were collected from patients attending the Endocrine and Diabetes Center at Al-Fayhaa Hospital, Basra, Iraq. The diagnosis of diabetes was made by specialized physicians in the field of diabetes. A total of 88 samples were collected, including the following: Patients group sixty blood samples were collected from women with diabetes. The samples were divided based on age categories as follows: women aged 45–54, totaling 29 samples and women aged 55–70, totaling 31 samples. The samples were also divided based on the duration of the disease as follows: women with a disease duration of 5–10 years, totaling 36 samples and women with a disease duration of 11–22 years, totaling 24 samples. Furthermore, the samples were categorized based on the severity of the disease determined by the specialist physician, according to the HbA1c value, as follows: women with moderate disease severity (HbA1c ranging from 7–9.9), totaling 32 samples and women with severe disease severity (HbA1c ranging from 10–14), totaling 28 samples.

**Healthy Group**

Blood samples were collected from healthy women after confirming that they did not have diabetes based on the HbA1c value measured using the VARIANT II BIO-RAD instrument. The age range of the healthy women was 45–70 years. The samples were randomly collected from both within and outside the specialized center.

**Preparation of Serum**

Five milliliters of venous blood were drawn using a medical syringe. The blood was then placed in a gel tube and left for 20 minutes. Subsequently, the tube was centrifuged at 3000 revolutions per minute for 10 minutes to obtain the serum. The serum was transferred to 1.5 mL Eppendorf tubes and stored at –20 °C in the freezer until further analysis.

**Estimation of the Concentration of Biomarkers in Patient and Healthy Serum**

The well-known Enzyme-Linked Immuno Sorbent Assay (ELISA) method was adopted using an American-made ELISA reader and diagnostic kits for the specific biomarkers in the current study. The kits were provided by MyBioSource, an American company, and were based on the enclosed datasheet for each biomarker, with a wavelength of 450 nanometers. The biomarkers included Endothelin-1 and Osteoprotegerin.

**Statistical Analysis**

Statistical analysis was performed on the data using the T-test to compare the means of patient samples and healthy samples at a significance level of ($P \leq 0.05$). The standard deviation (S.D.) was calculated using SPSS software, Version 21, from the Science Package for Social Statistical Analysis (Field, 2012).

## ■ RESULTS

### Endothelin-1 Biomarker

The results of the current study revealed a significant increase at a significance level of P≤0.05 in the concentration of Endothelin-1 among diabetic women (10.543) compared to healthy individuals (5.978). Furthermore, the results indicated a significant increase at a significance level of P≤0.05 in the concentration of Endothelin-1 in the early disease stage compared to the later stage. Additionally, a significant increase was observed in the high disease severity compared to the moderate severity, while no significant differences were found between the two age groups (Table 1).

### Osteoprotegerin biomarker

The statistical analysis revealed a significant increase at a significance level of P≤0.01 in the concentration of Osteoprotegerin in the serum of women with diabetes (952.796) compared to healthy women (492.507). Furthermore, the results indicated a significant increase at a significance level of P≤0.01 in the concentration of Osteoprotegerin in the first age group compared to the second age group. Additionally, a significant increase was observed in the second disease period and high disease severity compared to the first period and moderate disease severity (Table 2).

**Table 1**
**Concentration of Endothelin-1 in the serum of study samples**

| Variables | | No. | Mean±SD | P value |
|---|---|---|---|---|
| Participants | T2DM | 60 | 10.543**±1.934 | 0.0001 |
| | Healthy women | 28 | 5.978±1.718 | |
| Age (years) | 45–54 | 29 | 7.013±2.023 | 0.385 |
| | 55–70 | 31 | 6.549±2.079 | |
| Disease Duration | 5–10 | 36 | 7.842**±2.245 | 0.0001 |
| | 11–22 | 24 | 5.378±2.058 | |
| Disease Severity | HbA1c (7–9.9) | 32 | 3.798±1.95 | 0.0001 |
| | HbA1c (10–14) | 28 | 9.377**±2.633 | |

Note: ** significant difference at a significance level of P≤0.01.

**Table 2**
**Osteoprotegerin Concentration in Study Serum Samples**

| Variables | | No | Mean±SD | P value |
|---|---|---|---|---|
| Participants | T2DM | 60 | 952.796**±294.40 | 0.001 |
| | Healthy women | 28 | 492.507±149.785 | |
| Age (years) | 45–54 | 29 | 1078.21**±363.82 | 0.0001 |
| | 55–70 | 31 | 742.17±187.82 | |
| Disease Duration | 5–10 | 36 | 695±249.36 | 0.0001 |
| | 11–22 | 24 | 1282.04**±302.57 | |
| Disease Severity | HbA1c (7–9.9) | 31 | 676.71**±174.72 | 0.001 |
| | HbA1c (10–14) | 29 | 1193.42**±294.43 | |

Note: **significant difference at a probability level of P≤0.01.

## ■ DISCUSSION

The results of the current study showed a significant rise in the concentration of Endothelin-1 (ET-1) in the serum of diabetic patients compared to healthy individuals, Table 1 which is consistent with a study conducted by [20]. There are many explanations for this phenomena including notable elevation in ET-1 levels in diabetes may be attributed to hyperinsulinemia and elevated blood sugar levels, which lead to the activation of protein kinase C enzyme that stimulating the signaling pathway of mitogen-activated protein kinase (MAPK). This promote the expression of endothelin-converting enzyme (ECE), which convert big ET-1 into ET-1 inside the endothelial cells and subsequently elevate calcium channel activity and causes vasoconstriction of blood vessels [21].

Another reason it has also been demonstrated that insulin raise the ET-1 gene expression in endothelial cells, leading to the production of ET-1 in both smooth muscle cells and endothelial cells of blood vessels [22]. The hyperinsulinemia raise ET-1 levels in individuals with insulin resistance, which align with the elevated ET-1 levels in diabetes [23], due to activation of the PI3K-AKT-eNOS pathway which lead to production of high levels of nitric oxide (NO), which stimulate the peroxynitrite enzyme, which promote the production of ET-1. Additionally, it has been found that ET-1 weaken glucose uptake in skeletal muscles of insulin-resistant individuals, leading to the exacerbation of insulin resistance [24–26].

In this study, the high concentrations of ET-1 contribute to vascular dysfunction, ventricular and vascular remodeling, inflammation, and arrhythmias in heart failure. Also this elevation can cause vasoconstriction, resulting in elevated hypertenton, which lead to the workload which leading to myocardial stress, gradual damage, and heart failure. Therefore, ET-1 can be used as an independent predictor of survival in diabetes [27–29], enhance the production of inflammatory microglia cells [30], enhance the development of arterial sclerosis [31] by stimulate platelet accumulation and formation of fibrous plaques [32].

Additionally, the results revealed a statistically significant increase in endothelin concentration during the initial phase of the disease compared to the second phase. This finding is consistent with a study conducted by Liu et al. [33], which demonstrated that the duration of diabetes has an impact on serum ET-1 levels. During the shorter duration of the disease, ET-1 levels were elevated, while they decreased in longer time periods. The reason behind this phenomenon can be attributed to the vascular dysfunction induced by diabetes, leading to a reduction in the production of ET-1 [34].

Furthermore, a statistically significant elevation in endothelin levels was observed in the second disease severity compared to the first. This result align with a study by Sokolova et al. [35]. They concluded that the increase in disease severity in the second group in the current study may be attributed to the simultaneous rise in glucose levels in the plasma of diabetic patients. It is believed that the relationship between elevated HbA1c levels in diabetic patients and increased ET-1 concentration contributes to heightened oxidative stress occurring in the second group due to elevated glucose levels and, consequently, increased HbA1c levels [35].

As indicated by the findings of the current study, there is a significant increase in the concentration of Osteoprotegerin (OPG) in diabetes compared to healthy individuals, Table (2) and these findings were consistent with previous studies conducted by Olesen

et al. [36] and Chen et al. [37]. They said the elevated levels of OPG may be attributed to the severity of inflammation resulting from diabetes, by produce cytokines as tumor necrosis factor-alpha (TNF-α), that activate osteoclasts, stimulate the expression of RANKL by osteoblasts, leading to the binding of RANK to RANKL and activated through the RANKL-OPG-RANK system to bind to RANK and counteract the effects of RANKL, inhibiting the formation of bone resorbers, which lead to bone resorption. Consequently, inflammatory cytokines stimulate osteoblasts to express Osteoprotegerin as a protective mechanism against bone resorption, leading to an increased level of OPG in the blood [38], and this can be also observed with insulin resistance [39].

Authors concluded that OPG contribute to arteriosclerosis and cardiovascular diseases by amplifying the negative effects of inflammation and various traditional risk factors like dyslipidemia and hypertension resulting from diabetes. In addition, high level of OPG have a role in the progression of vascular complications [39].

Other explanation, the cytokines lead to an increased expression of OPG in vascular smooth muscle cells and endothelial cells [40], this is stimulated by inflammatory factors like T-lymphocytes and plasma cells, through the RANKL/RANK/OPG system that participate in the development of arteriosclerosis and inflammation-induced calcification [42]. The negative impact of elevated OPG levels exacerbate the inflammatory state induced by OPG since it is part of the TNF family of receptors, which in turn stimulate the expression of cytokines that lead to vascular damage [43].

Finally, the increase concentration of OPG stimulate the expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin in endothelial cells, promoting the adhesion of leukocytes [44]. Additionally, this stimulating the activity of metalloproteinase enzymes in vascular smooth muscle cells, which play a role in the degradation of certain components of the blood vessel walls, as collagen, elastin, and proteoglycans. These are lead to changes in the thickness and weakening of the vessel walls, creating spaces for fat accumulation and foam cell formation, that resulting in Arteriosclerosis [45].

This study focuses on predicting future cardiovascular disease (CVD) in diabetic patients through biomarker analysis. While our sample lacks current CVD, our aim is to identify predictors for future CVD events. Our study focuses on investigating the predictive utility of biomarkers for various cardiovascular diseases (CVDs) in patients with diabetes. While the current sample does not exhibit overt cardiovascular pathology, our aim is to identify biomarkers that may serve as predictors for future CVD events in this specific cohort.

We would like to highlight that throughout our discussion, we extensively reference the role of biomarkers in predicting multiple cardiovascular diseases. For instance, we discuss the impact of elevated endothelin-1 (ET-1) concentrations on vascular dysfunction, ventricular and vascular remodeling, inflammation, and arrhythmias in heart failure. Furthermore, we highlight ET-1›s association with increased arterial sclerosis, platelet accumulation, and formation of fibrous plaques, which are key factors in various cardiovascular conditions. However, our aim extends beyond merely reflecting biomarker levels in diabetic patients. We seek to elucidate the predictive value of these biomarkers for future cardiovascular diseases in this population. As such, we believe our study warrants classification as original research rather than a review. We appreciate your perspective.

## ■ CONCLUSION

Significantly elevation in both biomarkers, endothelin-1 and osteoprotegerin in patient with diabetes. These indicators could be play a role in predicting cardiovascular diseases before their occurrence in diabetes.

## ■ REFERENCES


1. Motawa IA, Aly MA, Metwally MM, Mohammed IN. Assessment of interleukin (8) in type 2 diabetes mellitus. *The egyptian journal of hospital medicine*. 2018;72(4):4403–4406.
2. Shehab A, Elkilany G, Singh RB, Hristova K, Chaves H, Cornelissen G, Otsuka K: Coronary risk factors in South West Asia. Editorial. *World Heart J.* 2015;7:21–30.
3. Schwartz GG, Bessac L, Berdan LG, Bhatt DL for ODYSSEY Outcomes Trial. Effect of alirocumab, a monoclonal antibody to PCSK9, on long-term cardiovascular outcomes following acute coronary syndromes: Rationale and design of the ODYSSEY Outcomes trial. *Amer Heart J.* 2014;168:682–689.
4. Einarson TR, Acs A, Ludwig C, Panton UH: Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007–2017. *Cardiovasc Diabetol*. 2018;17(1):83–83.
5. Hassan AM, Kata FS. The Role Of The Biomarkers Interleukin-18 And Transforming Growth Factor-Beta-1 In Predicting And Detecting Diabetic Nephropathy In Patients With Type 2 Diabetes. *Journal of Pharmaceutical Negative Results*. 2022;13(2):243–249.
6. Nakken B, Papp G, Bosnes V, Zeher M, Nagy G, Szodoray P. Biomarkers for rheumatoid arthritis: From molecular processes to diagnostic applications-current concepts and future perspectives. *Immunol. Lett.* 2018;189:13–18.
7. Abbas AJ, Kata FS. Relationship between Some Physiological Biomarkers in Obese Men and the Risk of Metabolic Syndrome. *Iranian Journal of War and Public Health*. 2022;14(4):385–390.
8. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res.* 2004;61(2):227–37.
9. Zhang C, Tian J, Jiang L, Xu L, Liu J, Zhao X, Feng X, Wang D, Zhang Y, Sun K. Prognostic Value of Plasma Big Endothelin-1 Level among Patients with Three-Vessel Disease: A Cohort Study. *J. Atheroscler. Thromb.* 2019;26:959–969.
10. Chen J, Chen MH, Guo YL, Zhu CG, Xu RX, Dong Q, Li JJ. Plasma Big Endothelin-1 Level and the Severity of New-onset Stable Coronary Artery Disease. *J. Atheroscler. Thromb.* 2015;22:126–135.
11. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Mickle DA. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation*. 2002;105(16):1890–1896.
12. Pernow J, Shemyakin A, Bohm F. New perspectives on endothelin-1 in atherosclerosis and diabetes mellitus. *Life Sci.* 2012;91:507–16.
13. Bernardi S, Bossi F, Toffoli B, Fabris B. Roles and clinical applications of OPG and TRAIL as biomarkers in cardiovascular disease. *BioMed Research International.* 2016;1752854.
14. Rochette L, Meloux A, Rigal E., Zeller M, Cottin Y, Vergely C. The role of osteoprotegerin in the crosstalk between vessels and bone: Its potential utility as a marker of cardiometabolic diseases. *Pharmacology & therapeutics.* 2018;182:115–132.
15. Venuraju SM, Yerramasu A, Corder R, Lahiri A. Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *Journal of the American College of Cardiology.* 2010;55(19):2049–2061.
16. Flyvbjerg A. Diabetic angiopathy, the complement system and the tumor necrosis factor superfamily. *Nat Rev Endocrinol.* 2010;6(2):94–101.
17. Ghaffari S, Yaghoubi A, Baghernejad R, Sepehrvand N, Sokhanvar S, Haghjou AG. The value of serum osteoprotegerin levels in patients with angina like chest pain undergoing diagnostic coronary angiography. *Cardiol J.* 2013;20(3):261–7.
18. Al-Akabi D, Kata FS. Effect of Non-alcoholic Fatty Liver Disease on Some of Bone Biomarkers in Men. *Open Access Macedonian Journal of Medical Sciences*. 2021;9(A):924–927.
19. Kiechl S. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation.* 2004;109:2175–80.
20. Ma Y, Tian T, Wang T, Wang J, Guan H, Yuan J, Qiao S. Predictive Value of Plasma Big Endothelin-1 in Adverse Events of Patients With Coronary Artery Restenosis and Diabetes Mellitus: Beyond Traditional and Angiographic Risk Factors. Frontiers in Cardiovascular Medicine. 2022;1298.
21. Oliver FJ, de la Rubia G, Feener EP. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. *J Biol Chem.* 1991;266:23251–23256.
22. Piatti P, Monti L D, Conti M, Baruffaldi L, Galli L, Phan C V, Pozza G. Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. *Diabetes*. 1996;45(3):316–321.
23. Sarafidis PA, Bakris GL. Insulin and endothelin: an interplay contributing to hypertension development? *The Journal of Clinical Endocrinology & Metabolism*. 2007;92(2):379–385.
24. Shemyakin A, Salehzadeh F, Böhm F, Al-Khalili L, Gonon A, Wagner H. Regulation of glucose uptake by endothelin-1 in human skeletal muscle in vivo and in vitro. *The Journal of Clinical Endocrinology and Metabolism.* 2010;95(5):2359–66.
25. Kawamura M, Heinecke J W, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. *Journal of Clinical Investigation.* 1994;94(2):771–778.
26. Dong, F, Zhang X, Wold L E, Ren Q, Zhang Z, Ren J. Endothelin-1 enhances oxidative stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells: role of ETB receptor, NADPH oxidase and caveolin-1. *British journal of pharmacology.* 2005;145(3):323–333.
27. Hulsmann M, Stanek B Frey B. Value of cardiopulmonary exercise testing and big endothelin plasma levels to predict short-term prognosis of patients with chronic heart failure. *J Am Coll Cardiol.* 1998;32(6):1695–1700.
28. Davie NJ, Schermuly RT, Weissmann N. The science of endothelin-1 and endothelin receptor antagonists in the management of pulmonary arterial hypertension: current understanding and future studies. *Eur J Clin Invest.* 2009;39(Suppl. 2):38–49.
29. Idris-Khodja N, Ouerd S, Mian MOR, Gornitsky J, Barhoumi T, Paradis P: Endothelin-1 overexpression exaggerates diabetes-induced endothelial dysfunction by altering oxidative stress. *Am J Hypertens.* 2016;29:1245–51.
30. Abdul Y, Jamil S, He L, Li W, Ergul A. Endothelin-1 (ET-1) promotes a proinflammatory microglia phenotype in diabetic conditions. *Can J Physiol Pharmacol.* 2020;98:596–603.
31. Wolf D, Ley K. Immunity and inflammation in atherosclerosis. *Circ Res.* 2019;124:315–27.

32. Schiffrin EL. Role of endothelin-1 in hypertension and vascular disease. *Am J Hypertens*. 2001;14:83S–89S.
33. Liu Y, Zhuang GB, Zhou XZ. HMBG1 as a Driver of Inflammatory and Immune Processes in the Pathogenesis of Ocular Diseases. *Journal of ophthalmology.* 2018;2018:1–8.
34. Ak G, Buyukberber S, Sevinc A, Turk HM, Ates M. The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with Type 2 diabetes mellitus. *Journal of Diabetes and its Complications.* 2001;15(3):150–157.
35. Sokolova LK, Yu BB, Pushkarev VV. The blood level of endothelin-1 in diabetic patients depending on the characteristics of the disease. *Mižnarodnij endokrinologìčnij žurnal*. 2020;16(3):204–208.
36. Olesen P, Ledet T, Rasmussen LM. Arterial osteoprotegerin: increased amounts in diabetes and modifi able synthesis from vascular smooth muscle cells by insulin and TNF-alpha. *Diabetologia.* 2005; 48:561–8.
37. Chen WJ, Rijzewijk LJ, van der Meer RW, Heymans MW, van Duinkerken E, Lubberink M. Association of plasma osteoprotegerin and adiponectin with arterial function, cardiac function and metabolism in asymptomatic type 2 diabetic men. *Cardiovasc Diabetol.* 2011;10:67.
38. Zhang J, Fu M, Myles D, Zhu X, Du J, Cao X, Chen YE. PDGF induces osteoprotegerin expression in vascular smooth muscle cells by multiple signal pathways. *FEBS letters.* 2002;521(1–3):180–1842.
39. Yaturu S, Rains J, Jain SK .Relationship of elevated osteoprotegerin with insulin resistance, CRP, and TNF-alpha levels in men with type 2 diabetes. *Cytokine*. 2008;44:168–71.
40. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures and mortality in elderly women. *J Clin Endocrinol Metab*. 2001;86:631–7.
41. Baud'Huin M, Duplomb L, Téletchéa S, Charrier C, Maillasson M, Fouassier M, Heymann D. Factor VIII-von Willebrand factor complex inhibits osteoclastogenesis and controls cell survival. *Journal of Biological Chemistry*. 2009;284(46):31704–31713.
42. Secchiero P, Corallini F, Pandolfi A, Consoli A, Candido R, Fabris B. An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. *Am J Pathol.* 2006;169:2236–44.
43. Collin-Osdoby P. Regulation of vascular calcification by osteoclast regulatory factors RANKL and osteoprotegerin. *Circ Res.* 2004;95:1046–57.
44. Zauli G, Corallini F, Bossi, F, Fischetti, F, Durigutto P, Celeghini C Secchiero P. Osteoprotegerin increases leukocyte adhesion to endothelial cells both in vitro and in vivo. *Blood, The Journal of the American Society of Hematology.* 2007;110(2):536–543.
45. Sandberg WJ, Yndestad A, Oie E, Smith C, Ueland T, Ovchinnikova O Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler Th romb Vasc Biol.* 2006;26:857–63.