Journal of Global Diabetes & Clinical Metabolism

Research Article

Influence of HbA1c on Nitric Oxide Level in Patients With Type 2 Diabetes Mellitus

This article was published in the following Scient Open Access Journal:
Journal of Global Diabetes & Clinical Metabolism
Received November 16, 2017; **Accepted** November 30, 2017; **Published** December 06, 2017

Dara Kutsyk*, Sklyarova H and Maksymets T

Danylo Halytsky Lviv National Medical University, Department of therapy №1 and medical diagnostic of postgraduate education, Ukraine

Abstract

Background: Type 2 diabetes mellitus is dangerous disease because of development micro- and macrovascular complications, which in most cases lead to death. Glycemia control, namely maintenance of HbA1c level < 7% is the main preventive measure to avoid these severe complications. Nitric oxide plays important role in vascular pathophysiology. But data about connection between HbA1c and nitric oxide are controversial. This study was undertaken to find out is there a link between nitrogen oxide and HbA1c.

Methods: The investigation included 50 patients with type 2 diabetes, who were divided into 2 groups: with HbA1c level <7% and HbA1c level >7%; and control group of 15 healthy subjects. Serum nitric oxide and other parametres were measured. Results: Negative correlation between HbA1c and nitric oxide was found. Level of nitric oxide was higher in patients with well controlled glycemia than in patients with bad controlled.

Conclusions: Production of nitric oxide is impaired in patients with type 2 diabetes, especially with bad controlled glycaemia. With increase in HbA1 serum nitric oxide decreases. Uncontrolled glycemia leads to impairment in nitric oxide metabolism what can be a reason of macro-and microvascular complications.

Keywords: Type 2 diabetes mellitus, HbA1c, Nitric oxide

Introduction

In 21 century type 2 diabetes (T2D) has become a global health and social problem in the whole world [1-4]. There are 382 million of people suffering from T2D, in 2030 there will be 592 million and in 2040 642 million [1,2]. Macrovascular (heart disease, stroke and peripheral vascular disease) and microvascular (retinopathy, neuropathy and nephropathy) diabetes -associated complications have adverse outcomes and in most cases lead to death [4-7]. Latter include lipid, blood pressure (BP) and glycaemia control [1-6]. American Diabetes Association (ADA) and International Diabetes Federation (IDF) allege to maintain optimal level of HbA1 below 7% and monitor it with 6 month interval [6,7]. People with good glycemic control have a lower risk of developing different complications and are prevented from diabetes-related death [7,8]. But for most patients it's difficult to achieve [9,10]. Permanent hyperglicemia, insulin resistance, hyperlipidemia lead to impairment of nitric oxide synthase (NOS), what is the reason of deacresed nitric oxid (NO) bioviability and pro-atheroghenic changes [11-14]. NO is the first member of gasotransmitters and plays an important role in pathogenesis of micro- and macrovascular complications [9]. It can be the earliest indicator of diseases, associated with oxidative stress, inflammation and endothelial dysfunction [6,9,11]. NO derives from L-arginin and this reaction is cathalized by NOS. There are three isoforms of NOS: endothelial nitric oxide synthase (eNOS), neuronal NOS (nNOS) and inducible (iNOS) [5]. In patients with diabetes vasodilation is impaired, as vessels become unresponsive to NO effect (this can be due to permanent hyperglycemia and adverse action of HbA1c) [11,12]. But the data about it are controversial. Studies show different results. This study was indicated to find out the influence and relationship between HbA1 and NO levels in T2D patients.

Materials and Methods

The investigation included 50 patients with T2D, who undergo treatment from march till october 2016 in Lviv Emergency Hospital. The study was approved by the ethical committee of the college. An informed consent was taken from each participant

***Corresponding author:** Dara Kutsyk, Department of therapy №1 and medical diagnostic of postgraduate education, Danylo Halytsky Lviv National Medical University, Pekarska Street, 69, L'viv, Lviv Oblast, Ukraine, Email: darmedter@gmail.com

before enrolling in the study. The Diagnosis of T2D was confirmed due to IDF criteria 2015. Exclusion criteria were first diagnosed diabetes, type 1 diabetes, isolated systolic hypertension, chronic liver diseases, neoplasms, chronic heart failure with mild range (40-49%) and reduced (<40%) ejection fraction (EF) (due to European Society Cardiology (ESC) criteria), chronic pulmonary disease, pneumonia, autoimmune diseases, coronary artery disease (CAD), patients who undergo dialysis, others endocrine diseases and smokers (due to World Health Organisation criteria > 1 cig/ month). All participants undergo clinical examination (including main anthropometric parametres- weight, height and waist circumference (WC)) case history and inventory questions. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m2). Systolic and diastolic blood pressure (SBP and DBP) was measured with the use of manual sphygmomanometer on the left arm in a sitting position after at least 10 min of rest. Blood samples for glucose, glycemic hemoglobin and lipids were taken after a 12-hour overnight fast. They were immediately centrifuged and analysed. NO was estimated by griess method after deproteinising the sample with 96% ethanol. All patients were divided into two groups: with well controlled glycaemia (HbA1 < 7%) and bad controlled glycaemia (HbA1> 7%). Control group consists of 25 absolute healthy subjects. Continuous characteristics were represented using means and standard deviations. Independent student t-test was used to compare differences between the different groups. Analysis of data was performed and expressed as (mean ± SD), quartile and ranges. Pearson correlation coefficient (r) was used for calculating the correlation of NO with HbA1c, serum sugar, creatinine, urea,uremic acid, WC, BMI, TG/HDL ratio, SBP and DBP, duration of disease and age in all patients. Level of statistical significance was set at P value < 0.05.

Results

Baseline characteristic of the patients is summarized in Table 1. The mean age of all subjects was 65,5 ± 10,1 years. There were 27 women and 23 men. The mean age of other parameters: WC 105,4 ± 11,6 cm, BMI 33,8 ± 4,8 kg/ m^2, SBP 145,5 ± 23,5 mmHg , DBP 93,7 ± 8,2 mmHg , total cholesterol 5,2 ± 1,5mmol/l, high density lipoproteins (HDL) 0,9 ± 0,2 mmol/l, low density lipoproteins (LDL) 2,8 ± 0,2 mmol/l, triglicerids (TG) 2,5 ± 0,9 mmol/l , TG/ HDL index 3,3 ± 0,5, fasting blood glucose (FBG) 9,4 ± 3,0 mmol/l, HbA1c 7,5 ± 2,0 %, creatinine 97,0 ± 23,5 µmol, urea 9,3 ± 2,1mmol/l, uremic acid 391,9 ± 118,9 mmol/l. Mean NO level in the group of T2D was 27,2 ±3,1, what was significantly higher compared to control group18,86±0,9 (p <0,001). In group with well controlled glycemia mean age of parametres were following: WC 106,1 ± 12,5 cm, BMI 34,0 ± 6,1 kg/ m2, SBP 162,5 ±24,0 mmHg , DBP 92,1 ± 7,5 mmHg , total choresterol 5,3 ± 1,3 mmol/l, HDL 0,9 ± 0,2 mmol/l, LDL 2,9 ± 0,1 mmol/l, TG 2,4 ± 0,1 mmol/l , TG/ HDL index 3,3 ± 0,1, FBG 8,5 ± 1,8 mmol/l, HbA1c 6,3 ± 2,8%, creatinine 87,7 ± 9,8 µmol, urea 7,8 ± 1,6 mmol/l , uremic acid 341,8 ± 104,8 mmol/l. In group with bad controlled glycemia mean age of parametres were following: WC 104,7 ± 10,1 cm, BMI 33,7 ± 2,91 kg/ m2, SBP 167,7 ± 24,0 mmHg , DBP 95,3 ± 8,6 mmHg, total choresterol 5,2 ± 1,6 mmol/l, HDL 0,9 ± 0,3 mmol/l, LDL 2,5 ± 0,5 mmol/l, TG 2,6 ± 0,8 mmol/l , TG/ HDL index 3,3 ± 0,1, FBG 10,3 ± 3,1 mmol/l, HbA1c 8,9 ± 3,1 %, creatinine 108,4 ± 30,1 µmol, urea 10,5 ± 1,2 mmol/l , uremic acid 452,7 ± 108,8 mmol/l (Table 2). A significant difference between two groups was revealed in creatinine and uremic acid (p<0,05).

	All T2D patients Mean ± S.D
Age (years)	65,5 ± 10,1
WC (cm)	105,4 ± 11,6
BMI (kg/ $m^{2)}$	33,8 ± 4,8
SBP (mmHg)	145,5 ± 23,5
DBP (mmHg)	93,7 ± 8,2
Total cholesterol (mmol/l)	5,2 ± 1,5
HDL (mmol/l)	0,9 ± 0,2
LDL (mmol/l)	2,8 ± 0,2
TG (mmol/l)	2,5 ± 0,9
TG/ HDL index	3,3 ± 0,5
FBG (mmol/l)	9,4 ± 3,0
HbA1c (%)	7,5 ± 2,0
Creatinine (µmol/l)	97,0 ± 23,5
Urea (mmol/l)	9,3 ± 2,1
Uremic acid (mmol/l)	391,9 ± 118,9

Table 1: Baseline characteristic of all patients.

	Well	Bad
Age (years)	65,7 ± 9,8	65,4 ± 10,9
WC (cm)	106,1 ± 12,5	104,7± 10,1
BMI (kg/ m2)	34,0 ± 6,1	33,7± 2,9
SBP (mmHg)	162,5 ± 24,0	167,7 ± 24,0
DBP (mmHg)	92,1 ± 7,5	95,3 ± 8,6
Total cholesterol (mmol/l)	5,3 ± 1,3	5,2 ± 1,6
HDL (mmol/l)	0,9 ± 0,2	0,9± 0,3
LDL (mmol/l)	2,9 ± 0,1	2,5 ± 1,5
TG (mmol/l)	2,4 ± 0,1	2,6 ± 0,8
TG/ HDL index	3,3 ± 0,1	3,3 ± 0,1
FBG (mmol/l)	8,5 ± 1,8	10,3± 3,1*
HbA1c (%)	6,3 ± 2,8	8,9 ±3,1*
Creatinine(µmol/l)	87,7 ± 9,8	108,4 ± 30,1*
Urea (mmol/l)	7,8 ± 1,6	10,5± 1,2
Uremic acid (mmol/l)	341,8 ± 104,8	452,7 ± 108,8*
NO (µmol)	28,6 ± 2,8*	25,3 ± 2,2*

Table 2: Characteristic of patients with well and bad controlled glycemia.

NO level in patients with well controlled glycemia was 28,6 ± 2,8µmmol, what was significantly higher than with bad controlled 25,3 ± 2,2 µmmol (p<0,05) (Table 2).

The strong negative correlation was revealed between HbA1c and NO in all T2D patients, showing that with increase of HbA1c, NO decreases (r= -0,399; p<0,01) (Figure 1).

Discussion

Increase of NO level, compared to controls proves that oxidative stress and inflammation play an important role in T2D. Endothelium releases NO in response to different inflammatory mediators and it results in vasodilation [9]. Such high raise of NO can be a compensatory mechanism or defensive response. The same results are shown in other studies [10-13]. In contrast various studies show decrease of NO [7,13]. Maybe it's because of duration of diabetes. Initially endothelium reveales NO in higher amount to protect organism from oxidative stress and inflammation. But therefore exhaustion occurs and NO decreases. That's why in later stages reduction is noticed. Another theory

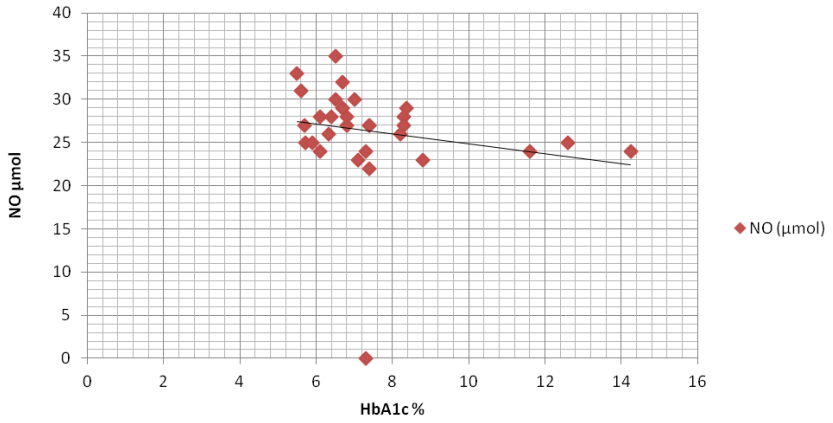

Figure 1: Correlation between HbA1c and NO.

is that bioavailability of NO is decreased in consequence NOS enzym occurs in the blood vessels of diabetic subjects, what leads to dysfunction of endothelium and excessive production of superoxide anion. Chronic hyperglycemia leads to congestion of the polyol pathway products and exhaustion of nicotinamide dinucleotide adenosine phosphate (NADPH) [1,4].

HbA1c is the indicator of T2D and the main proved predictor of complications. In ACCORD study treatment of hyperglycemia reduced macroalbuminuria, peripheral neuropathy and severity of visual disturbunces [3]. High glucose levels impair NO metabolism [11]. That is comfirmed in results, which show negative correlation between HbA1 and NO levels. The same results were found in other studies [14,15]. This finding is very important for future investigations. In group with well controlled glycemia, NO levels are higher, than in those with bad controlled. Maybe it's because of antidiabetic treatment. In normal conditions insulin stimulates the release of NO 20. Insulin resistance causes disruption of NO metabolism. Improvement of glycemic status leads to NO increase (this is shown in our results) and therefore prevention of future macro- and microvascular complications.

Difference in creatinine and uremic acid, which were higher in group with bad controlled glycemia proves that poor glycemic control leads to impairment of kidney's function and it can be due to intensification of oxidative stress (maybe via altered NO metabolism). But this is the question for future research.

Conclusions

Production of NO is impaired in patients with T2D, especially with bad controlled glycaemia. With increase in HbA1 serum NO decreases. This can be the main target for prevention vascular complication in T2D.

References

1. Baynest HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *J. Diabetes Metab*. 2015;6:1.
2. Eman Y. Mohammed, Dhia J, Al-Timimi, Suad Y, et al. Serum Nitric Oxide as a Biochemical Marker for Diabetic Nephropathy. *Journal of Advanced Biomedical & Pathobiology Research*. 2014;4:16.
3. Kumar S. Arvind Trivedi, Neetu Verma, Ajay Panwar, Pradeep Kumar. Evaluation of the Serum Levels of Nitric Oxide among Diabetic Patients and its Correlation with Lipid Profile as well as Oxidative Stress in North Indian Setting. *J Clin Diagn Res*. 2016;10(5):44-47.
4. Renuka NP, Naveen CR. Nitric Oxide in Diabetic Patients and its Relation with HbA1c. *International Journal of Pharmaceutical and Clinical Research.* 2014;6(2):146-148.
5. Faramar I, Timothy Craven, MaryAnn Banerji, Jan Basile, Jorge Calles,Effect of intensive treatment of hyperglycemia on microvascular complications of type 2 diabetes in ACCORD: a randomized trial. *Lancet.* 2010;376(9739):419-430.
6. Hermans MP, Jean-Charles Fruchart, Jean Davignon, et al. Residual Microvascular Risk in Type 2 Diabetes in 2014: Is it Time for or a Re-Think? A Perspective from the Residual Risk Reduction Initiative(R3i). *J Diabetes Metab*.2014;5:413.
7. Hsu Y. C, Lee P.H, Lei CC, Cheng Ho, Ya-Hsueh Shih. Nitric oxide donors rescue diabetic nephropathy through oxidative-stress- and nitrosative-stressmediated Wnt signaling pathways. J Diabetes *Investig*. 2015;6(1):24-34.
8. Manju M, Mishra S, Toora BD, Vijayakumar, R Vinod. Relationship between Glycosylated Hemoglobin, Serum Nitric Oxide and Mean Arterial Blood Pressure. *Int J Biomed Sci*. 2014;10(4):252-257.
9. Petimani S.M, Adake P, Babu S, Mahalaxmi S. Comparisiom of plasma glucose, serum ferritin, HbA1c and serum nitric oxide levels between diabetic and non- diabetic individuals: An indian scenario. *Int J Biomed Sci*. 2015;8:1.
10. Schneider MP, Ott C, Schmidt S. Stephanie Schmidt, Iris Kistner, Stefanie Friedrich,et al. Poor Glycemic Control Is Related to Increased Nitric Oxide Activity Within the Renal Circulation of Patients With Type 2 Diabetes. *Diabetes Care*. 2013;36(12):4071-4075.
11. American Diabetes Association. Classification and Diagnosis of Diabetes. *Diabetes Care.* 2015;38:8-16.
12. Tousoulis D, Papageorgiou N, Androulakis E, et al. Diabetes – Mellitus associated vascular impairment:novel circulating biomarkers and therapeutic approaches. *J Am Coll Cardiol.* 2013;62(8):667-676.
13. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The Role of Nitric Oxide on Endothelial Function. *Curr Vasc Pharmacol*. 2012;10(1):04-18.
14. Vincent MA, Montagnani M, Quon MJ. Molecular and physiologic actions of insulin related to production of nitric oxide in vascular endothelium. *Curr Diab Rep*. 2003;3(4):279-288.
15. Wimalawansa SJ. Preventing Long-Term Complications of Obesity, Type 2 Diabetes, and Metabolic Syndrome: Common Sense Approach. E*ndocrinol. Metab*. 2015;4 Syndr.2015;4:1.