



THE CORRELATION BETWEEN NITRIC OXIDE RADICALS AND IL-1 β PROTEIN IN NEWLY DIAGNOSED DIABETES MELLITUS TYPE 2

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ABSTRACT

Throughout life, the human body encounters numerous environmental and metabolic factors that generate reactive oxygen and nitrogen species (ROS/RNS). These reactive molecules can cause significant oxidative damage to cellular components. To maintain physiological balance, the body employs sophisticated endogenous antioxidant defense systems that neutralize these harmful free radicals and protect cellular integrity. This investigation sought to 1) examine the fundamental mechanisms of oxidative stress (OS) in diabetes mellitus type 2 (T2DM); 2) analyze the relationship between nitric oxide (NO) radicals and IL-1 β protein levels in recently diagnosed T2DM cases; and 3) compare the OS biomarkers between newly diagnosed and long-standing T2DM. The study enrolled 62 patients stratified into the long-standing T2DM group (n=42; disease duration >5 years) and the newly diagnosed T2DM group (n=20; diagnosis within the past year). All results were compared with healthy control subjects (n=33). Interleukin-1 beta (IL-1 β) protein concentrations were significantly elevated in newly diagnosed T2DM compared to controls (p < 0.001) and long-standing T2DM (p < 0.001). NO levels showed marked increases in recent-onset diabetics relative to the controls (p < 0.001) and long-standing diabetes patients (p < 0.001).

The study reveals that OS markers and inflammatory cytokines are already substantially elevated at the time of diabetes diagnosis. The pathophysiological processes underlying diabetes begin well before clinical manifestation. The concept of glycemic memory may explain these early biochemical changes. The OS biomarkers could serve as valuable early detection tools for prediabetic states. These findings highlight the importance of early OS-monitoring in at-risk populations and potential therapeutic interventions targeting inflammatory pathways.

Keywords: NO radicals, type 2 diabetes mellitus, IL-1 β protein,

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is fundamentally characterized by persistent hyperglycemia resulting from a complex interplay of genetic predisposition and environmental influences [1]. This metabolic disorder develops through two primary pathogenic mechanisms: a. impaired insulin secretion by pancreatic β -cells and b. insulin resistance in peripheral tissues, particularly adipose tissue, skeletal muscle, and the liver. The disease represents more than just a metabolic disturbance—it is now recognized as a chronic, low-grade inflammatory condition. This inflammatory state is evidenced by elevated circulating levels of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF- α), various interleukins (IL-1 β , IL-6), and adipokines (lipocalin-2, leptin, adiponectin, and resistin) [2]. These inflammatory molecules contribute significantly to the development and progression of insulin resistance. The adipose tissue, especially visceral fat, serves as a major endocrine organ that secretes these inflammatory factors, creating a systemic inflammatory milieu that exacerbates metabolic dysfunction. T2DM develops through multiple interrelated mechanisms. Over time, the insulin-producing beta cells in the pancreas gradually lose function, so they can no longer make enough insulin to regulate blood sugar [3]. At the same time, the body's tissues (such as muscle, fat, and liver cells) become less sensitive to insulin, a condition known as insulin resistance. Chronic, low-level inflammation also interferes with normal metabolism, and the hormonal signals from fat tissue (adipokines) become disturbed [4]. Together, these factors mean that T2DM presents with both metabolic problems (for example, high blood sugar and abnormal lipid levels) and systemic signs of inflammation. In other words, T2DM is a complex disorder involving both hormonal (endocrine) and immune-metabolic dysfunction. The inner lining of the blood vessels (the vascular endothelium) releases several bioactive molecules, including nitric oxide (NO) and endothelin-1. When blood glucose levels are elevated, the endothelium produces abnormally large amounts of NO. This excessive NO generation is closely linked to T2DM and its cardiovascular complications. NO is a very short-lived, highly reactive signaling molecule [5]. Under normal conditions, NO helps regulate blood vessel tone and generally keeps inflammation in check, but in disease

states, when it is overproduced, NO can act as a pro-inflammatory agent and contribute to vascular injury. Pro-inflammatory cytokines upregulate inducible NO synthase (iNOS) in immune cells such as macrophages and granulocytes, leading to a dramatic increase in NO production (up to 1000-fold). Excess NO in pancreatic tissue can impair insulin synthesis and release, potentially accelerating the onset of T2DM [5, 6].

This investigation sought to 1) examine the fundamental mechanisms of oxidative stress (OS) in T2DM; 2) analyze the relationship between NO radicals and IL-1 β protein levels in recently diagnosed T2DM cases; and 3) compare the OS biomarkers between newly diagnosed and long-standing T2DM.

MATERIALS AND METHODS

Subject Populations

This study included patients with newly diagnosed T2DM (n=20) who had no history of glucose-lowering or lipid-lowering therapies or antihypertensive therapies. The long-standing T2DM group (n=42) had a disease duration of 3 to 17 years. All results were compared with age-matched healthy subjects (n=33) without a history of type 2 diabetes, renal dysfunction, inflammation or medication, who were included as controls. T2DM was diagnosed based on generally accepted criteria for fasting blood glucose, random blood glucose and glycated hemoglobin.

Patients excluded from the study are: (a) undergoing surgery or medical treatment; (b) with a history of type 1 diabetes (T1DM), thyroid disease, stroke, or hemoglobinopathies; (c) pregnant; (d) with acute blood loss or blood transfusion; and (e) with a fasting time of less than eight hours. The study was conducted following the Declaration of Helsinki and approved by the Ethical Committee of the University Multidisciplinary Hospital, Active Treatment protocol code: 10-816; approval date 12 October 2019.

Measurement of Laboratory Parameters

The venous blood of fasting patients and healthy volunteers was collected in the morning after overnight fast-

ing for blood sugar and fasting insulinemia, glycated hemoglobin, lipid profile analysis. All collected samples for the electron paramagnetic resonance (EPR) study were studied immediately and then frozen at -80°C for the ELISA test. Fasting plasma glucose (FPG) concentrations and glycated hemoglobin (HbA1c%), representing an average measure of glycemic exposure over time, were measured as parameters for glycemic control.

Electron Paramagnetic Resonance (EPR)

All EPR measurements of all tested samples were conducted at room temperature (18–23°C) on an X-band EMXmicro spectrometer Bruker, Bremen, Germany, equipped with a standard Resonator. Quartz capillaries were used as sample tubes. The sample tube was sealed and placed in a standard EPR quartz tube (i.d. 3 mm), which was fixed in the EPR cavity. All EPR experiments were carried out in triplicate and repeated. Spectral processing was performed using Bruker WIN-EPR and SimFonia software 2021. Ex vivo EPR spectroscopy was used to study ROS formation in real time in the serum of patients and controls [7]. Laboratory measurement of NO radicals is extremely difficult due to their biochemical instability, short half-life, and sometimes very low concentration in biological fluids. EPR offers very high accuracy, using a spin trap, such as Carboxy-PTIO, by forming a stable adduct with the radical that exists long enough to be measured [8, 9]. The markers of OS were measured with ELISA kits following the manufacturer's instructions.

Statistical Analysis

Statistical analyses were conducted using Statistica 8 (StaSoft, Inc., Tulsa, OK, USA). Data are presented as means \pm standard error (S.E.). The correlation analysis were made by Scatter Diagram Correlation. Group differences were assessed by one-way ANOVA, followed by Fisher's least significant difference (LSD) post hoc test to identify specific intergroup differences, with $p < 0.05$ considered statistically significant.

RESULTS

Table 1. Clinical Characteristics of Subject Populations, statistical significance was set at $p < 0.05$, followed by an LSD post hoc test measured between the long-standing T2DM group vs the newly -diagnosed T2DM group. *HOMA-IR = (Fasting Glucose X Fasting Insulin) / 22.5.

Characteristics	control (n =33)	long-standing T2DM (n =42)	newly -diagnosed T2DM (n=20)	p
Age (years)	50.0 \pm 10.6	50.7 \pm 10.5	51.5 \pm 11.1	
Sex (% male)	41.3	25.5	28.4	
Family history of T2DM (% yes)	no	yes	yes	
Disease duration	-	10.7 \pm 7.3	0 \pm 12 (months)	
Body Mass Index BMI (kg/m²)	33 \pm 6	34 \pm 6	35 \pm 7	
Waist circumference (cm)	105 \pm 13	104 \pm 14	107 \pm 15	p = 0.1
2-h Oral Glucose Tolerance Test (2-h OGTT) mmol/L)	7.17 \pm 0.34	9.11 \pm 0.94	9.22 \pm 0.94	

Homeostasis model assessment of insulin resistance (HOMA-IR)*	1.65 ± 3.6	7.72 ± 4.4	14.1 ± 2.7	p = 0.001
Systolic blood pressure (mmHg)	120 ± 14	124 ± 14	129 ± 16	
Diastolic blood pressure (mmHg)	70 ± 9	78 ± 9	71 ± 10	
Urine Albumin Excretion UAE (mg/mmol)	1.2 ± 0.35	21.8 ± 1.78	27.3 ± 3.15	p = 0.001
Current smoker (n, %)	no	no	no	

Table 2. Oxidative stress biomarkers measure in T2DM and newly diagnosed T2DM, vs controls. Statistical significance was set at $p < 0.05$, followed by an LSD post hoc test measured between the long-standing T2DM group vs the newly -diagnosed T2DM group. # $p < 0.05$ vs controls.

Parameters	control (n =33)	long-standing T2DM (n =42)	newly -diagnosed T2DM (n=20)	p
OS biomarkers				
Malondialdehyd (MDA) $\mu\text{mol/ml}$	1.55 ± 0.07	2.87 ± 0.08	3.70 ± 0.12	p = 0.001
ROS (a.u.)	0.74 ± 0.03	2.33 ± 0.04	4.15 ± 0.07	p = 0.001
NO (a.u.)	14.51 ± 0.12	19.07 ± 0.11	38.32 ± 0.52	p = 0.001 #p=0.001
eNOS (pg/mL)	292.34 ± 5.34	460.87 ± 4.94	728.71 ± 18.43	p = 0.001
iNOS (pg/mL)	159.23 ± 1.42	245.65 ± 2.82	576.86 ± 22.32	p = 0.001
Lipocalin-2 (ng/mL)	69.42 ± 0.67	131.88 ± 1.51	161.82 ± 1.72	p = 0.001 #p=0.001
Protein carbonyl content (PC) $\mu\text{mol/mL}$	4.96 ± 0.05	8.28 ± 0.08	17.44 ± 0.16	p = 0.001
End Products of Advanced Glycosylation (AGEs) $\mu\text{mol/mL}$	3.25 ± 0.05	7.64 ± 0.04	15.65 ± 0.2	p = 0.001
Pro-inflammatory cytokines				
IL-6 pg/mL	5.57 ± 0.14	6.65 ± 0.22	12.79 ± 0.21	p = 0.001
TNF- α pg/mL	31.61 ± 0.57	42.68 ± 0.63	73.29 ± 0.67	p = 0.001
IL-1 β pg/mL	26.55 ± 1.29	66.25 ± 1.22	37.88 ± 2.01	p=0.00
Inflammatory parameters				
High-sensitivity C-reactive protein (hsCRP) mg/dL	0.33 ± 0.41	2.68 ± 0.51	2.89 ± 0.77	p=0.00
Glycemic parameters				
HbA1c (%)	4.2 ± 0.5	5.9 ± 0.5	9.1 ± 0.5	p=0.001
Fasting glucose (mmol/L)	5.31 ± 0.28	6.43 ± 0.89	10.94 ± 0.42	p=0.001
Fasting insulin ($\mu\text{U/mL}$)	7 ± 2.8	27 ± 1.6	29 ± 1.7	p=0.00
Serum creatinine ($\mu\text{mol/L}$)	63 ± 6.17	76 ± 9.16	86 ± 7.18	p=0.001
Total cholesterol (mmol/L)	4.17 ± 0.3	5.01 ± 0.7	7.47 ± 0.6	p=0.001
Triglycerides (mmol/L)	1.31 ± 0.15	2.13 ± 0.2	4.65 ± 0.2	p=0.001
Low-Density Lipoproteins (LDL) mmol/L	2.03 ± 0.11	2.72 ± 0.24	3.96 ± 0.2	p=0.00

Notes: *High HOMA-IR (≥ 2.56), elevated hsCRP (≥ 0.5 mg/dL).

The levels of OS biomarkers, NO and lipocalin-2 were significantly higher in patients with newly diagnosed type 2 diabetes than in healthy volunteers ($p < 0.001$) and patients with long-standing T2DM ($p < 0.001$). IL-1 β levels were higher in patients with T2DM compared to those with newly diagnosed diabetes ($p < 0.001$). NO radical levels were examined in relation to insulin resistance and the severity of inflammation. The analysis of the results shows that NO levels were significantly higher in patients with high HOMA-IR (Table 1) and elevated hsCRP (Table 2). Patients with newly diagnosed T2DM had significantly increased levels of biomarkers of OS (Table 2). Correlation analysis levels of NO radicals vs Lipocalin, $p=0.001$, $r=0.75$, and NO vs IL-1 β , $p=0.5$, $r = -0.617$. The study reported dramatically higher hs-CRP levels in both diabetic groups compared to controls. This suggests that elevated hs-CRP may also be an independent risk factor for worsening insulin resistance and obesity.

DISCUSSION

The study yielded several pivotal findings: (a) NO demonstrates a strong association with both proteinuria and insulin resistance; and (b) individuals newly diagnosed with T2DM exhibit significantly elevated NO levels. Indeed, NO concentrations were markedly higher in T2DM patients compared to healthy controls (see Table 2). Subgroup analysis within the diabetic cohort further revealed that both NO and hs-CRP levels were substantially elevated in newly diagnosed individuals relative to those with well-controlled (compensated) diabetes. These observations suggest a general upregulation of NO production in the T2DM context. However, inducible iNOS—a key enzymatic mediator of NO synthesis—exhibited concentration variability depending on the degree of glycemic control [10]. This may account for the heterogeneity observed in previous studies concerning NO levels in diabetic populations. The concurrent elevation of hs-CRP in newly diagnosed patients supports the hypothesis that increased NO production may be driven, at least in part, by underlying systemic inflammation. This notion is consistent with prior research implicating NO as a central regulator of inflammatory responses in T2DM. Of particular interest is the adipokine lipocalin-2, which possesses notable pro-inflammatory properties and is frequently elevated in obese individuals. Emerging evidence suggests a close regulatory relationship between NO and lipocalin-2 expression and enzymatic activation [11–13]. Specifically, NO has been shown to potently induce lipocalin-2 production [14]. In this study, lipocalin-2 levels were assessed relative to iNOS concentrations in T2DM patients. Under inflammatory conditions, NO appears to modulate lipocalin-2 expression within pancreatic β -cells and contributes to IL-1 β -mediated cytotoxic effects, including diminished glucose-stimulated insulin secretion and β -cell apoptosis. Furthermore, elevated levels of inflammatory mediators, including IL-1 β , TNF- α , IL-6, and hs-CRP, are frequently observed in obese individuals and those with metabolic syndrome. These biomarkers typically decline following weight reduction. Importantly, individuals with metabolic syndrome exhibit significantly

higher levels of IL-6, TNF- α , and hs-CRP compared to obese individuals without the syndrome, indicating that metabolic syndrome constitutes a more severe pro-inflammatory state [5, 6, 15]. Adipose tissue is now recognized as a major contributor to this inflammatory milieu, serving as a critical source of pro-inflammatory cytokines in both obesity and metabolic syndrome.

In individuals with T2DM and increased adiposity, chronic low-grade inflammation is a hallmark feature. Circulating IL-1 β levels are consistently elevated compared to healthy controls. IL-1 β impairs pancreatic β -cell function and promotes insulin resistance, accelerating disease progression. Conventional antidiabetic treatments often fail to significantly reduce IL-1 β levels [15]. A focused analysis revealed a distinct correlation between nitric oxide (NO) and IL-1 β depending on disease stage. In newly diagnosed T2DM, a strong positive correlation was observed ($r = 0.96$, $p < 0.02$), indicating acute inflammatory activation. This likely reflects early immune-mediated β -cell damage and systemic inflammation. IL-1 β induces iNOS, leading to increased NO production, which in turn reinforces inflammation and β -cell dysfunction through a feed-forward loop. In contrast, long-standing T2DM showed a weaker, non-significant correlation between NO and IL-1 β ($r = 0.9$, $p = 0.3$). This may result from chronic adaptation to hyperglycemia, long-term pharmacotherapy, or altered inflammatory signaling. Compensatory mechanisms such as iNOS downregulation or increased antioxidant activity may also play a role. Prolonged inflammation may desensitize cells, reducing cytokine and NO responses over time. These findings highlight the dynamic nature of inflammation in T2DM. In early stages, IL-1 β -driven NO production contributes to disease onset, while in advanced stages, this interaction becomes less consistent. Understanding these temporal patterns may guide the development of stage-specific anti-inflammatory and antioxidant therapies.

Nevertheless, certain pharmacologic agents exhibit dual glucose-lowering and anti-inflammatory properties. For instance, thiazolidinediones suppress the expression of pro-inflammatory cytokines such as IL-6 and TNF- α in multiple tissues, including plasma, hepatic, and adipose compartments—effects that are particularly pronounced in obese individuals with T2DM. Similarly, metformin has been shown to attenuate the secretion of pro-inflammatory cytokines and improve the overall inflammatory profile. It also reduces the neutrophil-to-lymphocyte ratio, a well-established marker of systemic inflammation and immune dysregulation. Moreover, insulin therapy exerts anti-inflammatory effects by downregulating IL-6 and IL-1 β production, partially via inhibition of the nuclear factor kappa B (NF- κ B) signaling pathway, a key transcriptional regulator of inflammatory gene expression.

CONCLUSION

These findings highlight the intricate relationship between NO, systemic inflammation, and metabolic dysfunction in T2DM. Elevated NO levels in newly diagnosed T2DM patients suggest a strong association with the early inflammatory state of the disease. Additionally, the inter-

play between NO, lipocalin-2, and pro-inflammatory cytokines like IL-1 β underscores the immune-metabolic nature of T2DM. While controlling blood glucose remains a fundamental therapeutic goal, targeting the inflammatory component of the disease may offer added benefits. Anti-inflammatory agents such as thiazolidinediones, metformin, and insulin not only improve metabolic parameters but also reduce pro-inflammatory cytokines, potentially slowing disease progression and preventing complications. Integrating inflammation-modulating strategies into standard diabetes care could represent a more comprehensive approach to managing this complex disorder.

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