

TO
THE CHAIR OF THE SCIENTIFIC JURY,
APPOINTED BY ORDER No. P-109-300/21.08.2026r.
OF THE RECTOR OF MEDICAL UNIVERSITY – VARNA

REVIEW

by Professor Tsvetelina Valentinova Miteva, MD, PhD

**Head of the Department of General Medicine, Forensic Medicine and
Deontology**

Faculty of Public Health

Medical University – Pleven

for the procedure for the acquisition of the educational and scientific degree
"Doctor" in the doctoral programme "General Medicine", professional field 7.
Health Care and Sport, professional field 7.1. Medicine

Author: Dr. Nikolay Alexandrov Bekyarov

Title: Evaluation and role of certain markers of endothelial dysfunction and
oxidative stress in outpatients with metabolic syndrome

Scientific supervisor: Professor Valentina Madzhova, MD, PhD, Medical
University "Prof. Dr. Paraskev Stoyanov" – Varna

1. General presentation of the procedure and the doctoral candidate

The dissertation submitted to me for review is intended for consideration by a
scientific jury appointed by Order No. P-109-300/21.08.2026r. of the Rector of
Medical University – Varna. I have received all the documents required by the
procedure.

Dr. Nikolay Bekyarov graduated from the Medical University "Prof. Dr.
Paraskev Stoyanov" in Varna with a Master's degree in Medicine in 2004, and
subsequently obtained a Master's degree in Health Management from the same
university in 2014. Following his specialization training, he qualified as a specialist
in Neurology.

His professional career began as a staff physician in the Neurology Department of the Multiprofile Hospital for Active Treatment (MHAT) "Tervel" ; later he worked in the Emergency Department of MHAT "Dobrich". Currently, Dr. Bekyarov serves as a staff physician at MHAT "Dobrich" while simultaneously providing neurological consultations at the Medical Center in Dobrich - "Viva Phoenix". In 2023, he enrolled as a part-time doctoral student in the General Medicine program at the Department of General Medicine in Medical University "Prof. Dr. Paraskev Stoyanov" - Varna .

2. General characteristics of the dissertation

The dissertation comprises 103 pages, including the bibliography, and is illustrated with 4 tables and 33 figures. Its structure adheres to generally accepted standards and includes all necessary sections: introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusions, contributions, and bibliography.

The aim of the dissertation is to investigate and evaluate the role of certain markers of endothelial activation and oxidative stress in outpatients with metabolic syndrome. In line with this objective, eight tasks have been formulated in a logical sequence:

1. To investigate plasma levels of asymmetric dimethylarginine (ADMA), a marker of endothelial dysfunction
2. To investigate malondialdehyde (MDA) as a marker of oxidative stress in individuals with metabolic syndrome
3. To examine lipid profile parameters, glucose and uric acid levels, and body mass index (BMI)
4. To investigate correlations of ADMA and MDA with lipid profile parameters and atherogenic indices
5. To investigate the expression of transcription factors Nrf2 and NF- κ B, as well as HO-1, in PBMCs of individuals with metabolic syndrome
6. To examine the relationship between ADMA, HO-1, Nrf2, and NF- κ B in individuals with metabolic syndrome

7. To investigate and evaluate changes in carotid intima-media thickness (CIMT) with age in individuals with metabolic syndrome
8. To evaluate the relationship between CIMT, ADMA, HO-1, NF- κ B, and Nrf2 in individuals with metabolic syndrome

3. Relevance of the topic

The topic of asymmetric dimethylarginine (ADMA) and malondialdehyde (MDA) as markers of oxidative stress and endothelial dysfunction in individuals with metabolic syndrome within outpatient practice remains highly relevant and scientifically significant, while simultaneously evolving from purely fundamental scientific inquiry toward an assessment of its clinical value.

The scientific and theoretical relevance of this topic is defined by the central role of oxidative stress in metabolic syndrome, where hyperglycemia, insulin resistance, and visceral obesity lead to the overproduction of reactive oxygen species (ROS). Malondialdehyde (MDA) is one of the most thoroughly studied end-products of polyunsaturated fatty acid peroxidation; it directly reflects the extent of cellular damage and membrane destruction caused by oxidative stress. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS). Elevated ADMA levels lead to reduced nitric oxide (NO) production, accelerate atherogenesis, and impair vasodilation. Oxidative stress (high MDA) inhibits the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which is responsible for the degradation of ADMA. This leads to the secondary accumulation of ADMA, further coupled eNOS uncoupling, and subsequent, even more intense ROS generation. The combination of MDA and ADMA allows for the assessment of damage at the subclinical stage of cardiovascular involvement—long before the onset of overt clinical events (such as heart attack or stroke) or morphological changes (plaques, altered intima-media thickness).

These processes have clinical significance, given that patients with metabolic syndrome fall into a high-risk category, and general practice serves as the primary setting for the prevention of type 2 diabetes and cardiovascular diseases. Measuring markers such as ADMA and MDA helps stratify patients into subgroups with low versus extremely high oxidative or vascular risk. Furthermore, MDA and ADMA levels are responsive to pharmacological interventions and lifestyle changes; consequently, they can be used to monitor therapeutic efficacy during treatment with statins, ACE inhibitors, SGLT2 inhibitors, and antioxidants, as well as during dietary and physical activity interventions.

4. Familiarity with the problem

The doctoral candidate demonstrates a sound understanding of the subject matter. A total of 269 literature sources, covering the last 20 years of research, have been analyzed. An extensive body of both national and international literature has been reviewed. Existing data on endothelial dysfunction and the role of oxidative stress indicate that markers of endothelial dysfunction remain insufficiently studied, furthermore, elevated levels of total cholesterol, triglycerides, glucose, and certain pro-inflammatory markers do not constitute sufficient criteria for diagnosing endothelial dysfunction or assessing cardiovascular risk.

While there is some evidence regarding the role of ADMA as an eNOS inhibitor and a marker of endothelial dysfunction, data concerning the role of the enzyme heme oxygenase-1 (HO-1) as a protective agent against oxidative stress and inflammation are lacking. Information is limited regarding the role of the redox-sensitive transcription factors NF-kB and Nrf2 as regulators of the inflammatory response and antioxidant defense in the pathogenesis of endothelial dysfunction associated with metabolic syndrome. There is also a lack of information concerning the interrelationships between the ROS-regulated transcription factors NF-kB and Nrf2, HO-1, and ADMA. The reviewed literature is extensive and presented in an analytical and in-depth manner, demonstrating the candidate's well-informed status and ability to conduct a competent analysis of the source material.

5. Research methodology

Several methods were employed in conducting the study:

1. Questionnaire-based – for gathering the information on demographics (gender, age, place of residence), education level, presence of metabolic syndrome components and other cardiovascular risk factors (arterial hypertension, type 2 diabetes, obesity), family history, genetic predisposition to vascular diseases, presence of chronic cardiovascular diseases (CVD), dyslipidemia, lifestyle, and physical activity data.

2. Clinical – for measuring height, waist circumference (WC), and body mass index (BMI).

3. Laboratory and biochemical – for venous blood glucose measurement and serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides; plasma concentrations of

asymmetric dimethylarginine (ADMA); and lipid peroxidation, analyzed via malondialdehyde (MDA) levels.

4. Genetic – collection of peripheral blood mononuclear cells (PBMC) for gene expression analysis.

5. Functional assessments – Doppler sonography to calculate carotid intima-media thickness (CIMT).

6. Statistical – statistical processing of results using ANOVA, Student's paired t-test (95% confidence interval), and correlation analysis to determine the correlation coefficient (r).

A total of 83 individuals (63 women, 20 men) aged 30 to 64 years were included in the study. The participants were divided into two groups: Group 1, consisting of individuals with newly diagnosed metabolic syndrome (n = 63), and Group 2, consisting of clinically healthy individuals (control group) (n = 20). Subjects in the metabolic syndrome group were selected according to NCEP-ATP III criteria.

6. Original results

The study results demonstrate significantly higher plasma ADMA levels in individuals with metabolic syndrome compared to the control group. They also show elevated plasma MDA levels in the patients studied. Correlations were established between MDA and lipid markers, as well as between MDA and BMI, within the metabolic syndrome group. This suggests a potential role for ADMA and MDA as predictive biomarkers for endothelial dysfunction and cardiometabolic risk.

The study reveals increased HO-1 expression in PBMCs, highlighting the significance of the transcription factor Nrf2—which plays a key role in endothelial protection—and NF- κ B, a transcription factor mediating the inflammatory response in the pathogenesis of endothelial dysfunction associated with metabolic syndrome.

The data indicate increased carotid intima-media thickness (CIMT) in individuals with metabolic syndrome and a positive correlation between CIMT and age; furthermore, elevated NF- κ B expression was found to correspond with increased plasma levels of ADMA and MDA.

The discussion of the results is conducted at a high academic level, drawing comparisons with various international findings and establishing logical cause-and-effect relationships. The doctoral candidate demonstrates strong skills in data interpretation, as well as in the analysis and synthesis of the collected information.

7. Contributions of the scientific work, in theoretical and practical terms

The study contributes to enriching the concept of early "biomarkers of vascular aging" and the personalized approach to treating metabolic syndrome in primary outpatient care. Its contributions can be categorized into theoretical and practical aspects:

Scientific-theoretical contributions:

A. Original contributions:

For the first time in national outpatient practice, a simultaneous study was conducted on the genetic/protein expression of the transcription factors Nrf2 and NF-kB, in conjunction with the cytoprotective enzyme HO-1, in peripheral blood mononuclear cells (PBMC) of individuals with metabolic syndrome. The pathogenetic interrelationship between cellular antioxidant capacity (Nrf2/HO-1), pro-inflammatory signaling (NF-kB), plasma markers of oxidative stress (MDA), endothelial dysfunction (ADMA), and structural vascular changes (CIMT) was elucidated. A synergistic effect between advancing age and disrupted redox balance on the accelerated thickening of the intima-media complex (CIMT) in patients with metabolic syndrome was demonstrated.

B. Confirming contributions:

A significant increase in malondialdehyde (MDA) and asymmetric dimethylarginine (ADMA) levels in individuals with metabolic syndrome has been confirmed, as has their strong positive correlation, reflecting the link between lipid peroxidation and the inhibition of endothelial NO. The high diagnostic value of calculated atherogenic indices (TC/HDL and LDL/HDL) for assessing cardiovascular risk in the context of atherogenic dyslipidemia associated with metabolic syndrome has also been confirmed.

Scientific-practical (applied) contributions:

A. An enhanced biochemical panel incorporating ADMA and MDA has been proposed for the detection of early oxidative and endothelial stress in outpatients with metabolic syndrome, prior to the onset of overt organ damage or plaque formation.

B. The demonstrated correlation between CIMT and markers of oxidative stress/antioxidant defense justifies the combined use of carotid artery ultrasound and redox markers to identify high-risk patients exhibiting accelerated vascular aging.

C. An additional method for monitoring therapeutic efficacy has been presented. The markers Nrf2, HO-1, ADMA, and MDA emerge as sensitive biological targets for the subsequent assessment of the efficacy of pharmacological agents (e.g., statins, SGLT2 inhibitors, ACE inhibitors) and lifestyle changes during outpatient follow-up.

Recognized limitations and practical challenges include:

- Laboratory standardization: The determination of MDA and ADMA requires specialized equipment and standardized reference ranges that have not yet been routinely implemented in standard outpatient laboratories.
- Financial and analytical accessibility: High-precision methods for these measurements are costly and are primarily used in research centers.

8. Assessment of publications and the doctoral candidate's personal contribution

Dr. Nikolay Bekyarov's publications present the key results of the conducted research. The doctoral candidate has submitted three full-text papers published in peer-reviewed international journals with a high impact factor.

The autoreferat (dissertation summary) meets the regulatory requirements, is correctly structured, and fully reflects the content, methods, results, conclusions and contributions of the dissertation.

Conclusion

The dissertation of Nikolay Bekyarov meets the requirements of the Act on the Development of the Academic Staff of the Republic of Bulgaria (ADASRB), the Regulations for the Implementation of the ADASRB, and the Regulations for the Development of the Academic Staff of Medical University – Varna.

It presents scientific and applied results, aligns with the latest trends in the search for molecular targets and predictive biomarkers for the early prevention of cardiometabolic diseases, and demonstrates that Dr. Nikolay Bekyarov possesses the qualities to conduct independent scientific research in the field.

In view of the above, I consider that the submitted dissertation meets all regulatory requirements, and I support the conferral of the scientific and educational degree "Doctor" on Dr. Nikolay Bekyarov.

9.10.2026

Reviewer:

Заличено на основание чл. 5,
§1, б. „Б“ от Регламент (ЕС)
2016/679

Prof. Tsvetelina Valentinova Miteva, MD, PhD