

REVIEW

by Prof. Radost Spiridonova Asenova, MD, DSc
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Member of the Scientific Jury and reviewer, appointed by Order No. R-109-300/21.08.2026 of the Rector of Medical University “Prof. Dr. Paraskev Stoyanov” – Varna, pursuant to Minutes No. 1/28.08.2026.

REGARDING: the dissertation entitled “Assessment and Role of Certain Markers of Endothelial Dysfunction and Oxidative Stress in Outpatients with Metabolic Syndrome”, submitted for the award of the educational and scientific degree “Doctor” (PhD) in field of higher education 7. Health Care and Sport, professional field 7.1. Medicine, doctoral programme “General Medicine”

Author: Dr. Nikolay Alexandrov Bekyarov

Scientific supervisor: Prof. Valentina Madjova, MD, PhD

General overview of the procedure and the doctoral candidate

This review has been prepared pursuant to Order No. R-109-300/21.08.2026 and the decision recorded in Minutes No. 1/28.08.2026. The materials submitted for assessment include all the necessary documents relating to the procedure and allow a comprehensive assessment of the research, the candidate’s personal contribution and professional training.

Dr. Nikolay Alexandrov Bekyarov graduated in medicine from Medical University – Varna in 2004. He obtained a specialist qualification in Neurology in 2015 and a Master’s degree in Health Management. His professional experience includes work in neurology and emergency departments. Since 2007, he has worked as a neurology specialist at the Multiprofile Hospital for Active Treatment – Dobrich; he also provides outpatient neurological care. His clinical background, including training in ultrasound examination of cerebral vessels, electromyography and electroencephalography, is directly relevant to early vascular damage and cardiovascular risk assessment.

The candidate is enrolled in the doctoral programme “General Medicine”. His submitted biography demonstrates a sustained interest in vascular pathology, metabolic syndrome and the early detection of subclinical vascular changes. This professional background is appropriate for an interdisciplinary study linking general medicine, neurology, laboratory medicine, molecular medicine and vascular ultrasound diagnostics.

Relevance and significance of the topic

Metabolic syndrome is among the most significant contemporary medical and social problems because of its high prevalence, early onset and established association with type 2 diabetes mellitus, cardiovascular disease and cerebrovascular events. It requires comprehensive risk assessment, active follow-up and early preventive intervention in general practice.

Endothelial dysfunction precedes the clinical manifestation of atherosclerosis and provides an important link between metabolic disturbances, oxidative stress, inflammation and structural vascular damage. The search for reliable markers that identify early pathological changes before irreversible vascular complications develop is therefore of considerable scientific and practical importance.

The dissertation provides a comprehensive assessment of ADMA, MDA, HO-1, the redox-sensitive transcription factors Nrf2 and NF-κB, and carotid intima-media thickness (CIMT). Comparing biochemical, molecular and structural parameters in outpatients with newly diagnosed metabolic syndrome is a relevant approach, allowing investigation of the balance between mechanisms of endothelial damage and antioxidant defence. The topic is particularly

relevant to general medicine, where early identification of high-risk patients and timely prevention are key tasks.

Structure and characteristics of the dissertation

The dissertation comprises 104 pages and is structured into nine main sections: introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusions, contributions, publications related to the dissertation, and references. The bibliography contains 281 references, demonstrating an extensive examination of the scientific literature.

The literature review systematically examines endothelial dysfunction and vascular oxidative stress, the role of free radicals, redox signalling and transcription factors, the significance of ADMA, the protective function of HO-1, and the role of CIMT as a marker of subclinical atherosclerosis and cardiovascular risk. The author traces the relationships between these mechanisms and the components of metabolic syndrome and explains the need for their comprehensive investigation.

The review is informative and covers the main pathophysiological mechanisms relevant to the topic. The effort to present both the damaging mechanisms - reduced nitric oxide bioavailability, lipid peroxidation and pro-inflammatory signalling - and the compensatory Nrf2/HO-1 antioxidant pathways deserves a positive assessment.

Aim and objectives

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. Eight objectives have been formulated: to determine plasma ADMA and MDA levels; assess the lipid profile, glucose, uric acid, BMI and waist circumference; analyse correlations with lipid parameters and atherogenic indices; investigate Nrf2, NF- κ B and HO-1 expression in peripheral blood mononuclear cells; and assess the relationships between these markers, age and CIMT.

The objectives are logically aligned with the aim and establish a coherent analytical framework, progressing from traditional cardiometabolic parameters to biochemical and molecular mechanisms and the structural marker of subclinical atherosclerosis.

Materials and methods

The study was approved by the Scientific Ethics Committee of the Research Council of Medical University - Varna (Minutes No. 86/26.09.2019). It included 83 participants aged 30 - 64 years: 63 women and 20 men. Participants were divided into a group of 63 outpatients with newly diagnosed metabolic syndrome and a control group of 20 clinically healthy individuals. Diagnosis was based on the NCEP-ATP III criteria, and participants with metabolic syndrome were not receiving treatment. Inclusion and exclusion criteria are specified.

A broad range of methods was used. A questionnaire collected demographic data and information on the components of metabolic syndrome, other cardiovascular risk factors, family history and physical activity. Anthropometric measurements and standard clinical laboratory tests for glucose and the lipid profile were performed, and the Castelli I and II atherogenic indices were calculated. Plasma ADMA concentration was determined using a locally validated ELISA method, and lipid peroxidation was assessed through MDA levels. The molecular genetic component included isolation of peripheral blood mononuclear cells and gene expression analysis. Structural vascular assessment used B-mode carotid ultrasonography, with three standardised measurements on each side and calculation of mean CIMT. The combination of clinical, laboratory, molecular and imaging methods is a major strength of the study.

Statistical analysis included ANOVA, Student's t-test and correlation analysis. Results are presented as mean $\pm$ standard error, with a statistical significance threshold of $p < 0.05$. Excel and GraphPad Prism were used. These methods allow the principal comparisons and analysis of relationships.

Results and discussion

The results are organised into three interrelated areas. The first analyses ADMA, MDA and traditional metabolic parameters. Higher plasma ADMA levels were found in participants with metabolic syndrome than in controls, together with a significant increase in MDA. Participants with metabolic syndrome had adverse lipid profiles and atherogenic indices, and higher glucose, BMI and waist circumference values. Correlations of ADMA with triglycerides, HDL cholesterol and the TC/HDL and LDL/HDL indices are presented, as are associations of MDA with individual lipid parameters.

These results support the view that endothelial dysfunction and oxidative stress are early, interrelated processes in metabolic syndrome. The positive association between ADMA and MDA is particularly important, linking impaired NO-dependent endothelial function with lipid peroxidation. The observed associations with atherogenic indices complement traditional risk assessment and provide a rationale for larger studies of the prognostic value of these markers.

The second area assesses Nrf2, NF- κ B and HO-1 expression in peripheral blood mononuclear cells. The data indicate activation of both antioxidant defence mechanisms and pro-inflammatory signalling in metabolic syndrome. Analysis of the Nrf2/HO-1 axis is scientifically significant, reflecting the compensatory response to oxidative stress, whereas NF- κ B characterises the inflammatory component of vascular damage. Their joint assessment with ADMA and MDA is one of the strengths of the dissertation.

The third area examines CIMT and its associations with age and redox balance. Higher CIMT values were found in participants with metabolic syndrome, increasing with age. Comparing this structural marker with ADMA, MDA, HO-1, Nrf2 and NF- κ B allows functional and molecular changes to be considered in the context of subclinical atherosclerosis.

The discussion is detailed and compares the findings with published international data. The author demonstrates a sound understanding of the pathophysiology of endothelial dysfunction, oxidative stress and vascular ageing.

Clinical applicability should be interpreted cautiously. The cross-sectional study cannot establish causal relationships or prognostic value. The small control group, uneven sex distribution and limited sample size restrict the generalisability of the findings. At this stage, ADMA, MDA and molecular markers cannot replace established cardiovascular risk assessment. Nevertheless, the results provide a sound basis for prospective studies and the development of combined models for early risk stratification.

Conclusions, contributions and practical significance

Eight conclusions have been formulated, largely deriving from the results and corresponding to the objectives. They summarise elevated ADMA and MDA levels, the adverse metabolic profile, activation of Nrf2, NF- κ B and HO-1, higher CIMT values, and associations with age and redox imbalance. The conclusion that ADMA and MDA may be useful diagnostic markers should be regarded as a promising hypothesis requiring confirmation in larger independent cohorts and assessment of sensitivity, specificity and incremental prognostic value.

The contributions are classified as original, confirmatory and applied scientific contributions. I consider the most significant to be the comprehensive investigation of the relationships between ADMA, MDA, HO-1, Nrf2 and NF- κ B; their comparison with traditional metabolic parameters and atherogenic indices; and the integration of biochemical and molecular markers with CIMT as a structural indicator of subclinical vascular damage. This approach advances understanding of the balance between endothelial damage, inflammation and antioxidant defence in metabolic syndrome.

The applied scientific contributions should be understood as identifying potential and future directions. Nevertheless, the study conveys a clear practical message: patients with metabolic syndrome should be identified and followed up early, and assessment of traditional risk factors may be enhanced by investigating mechanisms of endothelial damage and subclinical atherosclerosis.

Publications and personal contribution

The dissertation lists three publications related to the topic. They present the main research areas: the relationship of ADMA and oxidative stress with endothelial dysfunction; associations between Nrf2, HO-1 and NF- κ B expression, plasma ADMA and oxidative stress; and age-related changes in CIMT in relation to redox balance indices in metabolic syndrome. Two publications appeared in international journals with an impact factor.

Dr. Bekyarov's personal contribution is evident in his involvement in participant selection and clinical assessment, data collection and systematisation, vascular assessment, and interpretation of the results in the context of early vascular damage. The interdisciplinary nature of the project entails teamwork with clinical laboratories and researchers in molecular medicine and diagnostic imaging. The dissertation demonstrates the candidate's ability to contribute fully to the planning, conduct and scientific presentation of a comprehensive clinical study.

Critical comments and recommendations

I have no critical comments.

Conclusion

Dr. Nikolay Alexandrov Bekyarov's dissertation is a relevant, independent and interdisciplinary scientific study of markers of endothelial dysfunction, oxidative stress and subclinical vascular damage in outpatients with metabolic syndrome. It uses a comprehensive methodological approach combining clinical, biochemical, molecular genetic and ultrasound investigations. The findings advance understanding of the relationships between cardiometabolic risk, redox imbalance, inflammatory signalling and early structural vascular changes. The dissertation contains scientific and applied scientific contributions and demonstrates the author's preparedness for independent research.

In my opinion, the dissertation meets the requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria, its implementing regulations and the applicable internal regulations of Medical University - Varna.

On these grounds, I give the dissertation a positive assessment and confidently recommend that the esteemed Scientific Jury award Dr. Nikolay Alexandrov Bekyarov the educational and scientific degree "Doctor" (PhD) in field of higher education 7. Health Care and Sport, professional field 7.1. Medicine, doctoral programme "General Medicine".

09.10.2026

Review prepared by:

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