

STATEMENT

МЕДИЦИНСКИ УНИВЕРСИТЕТ
ПЛОВДИВ
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By assoc. prof. Milena Georgieva Velizarova, MD, PhD

Head of the Department of Clinical laboratory, Faculty of Medicine, Medical University-Sofia

Subject: PhD thesis of Dr. Desislav Grozev Tomov, presented for obtaining a PhD degree
Professional field 7.1. Medicine, Doctoral program "Medical biology"

Author: Dr. Desislav Grozev Tomov

Type of Doctoral training: independent training

Department: Medical Biology, МФ, Faculty of Medicine, Medical University-Plovdiv

PhD thesis title: „Oxidative stress determination using liquid chromatography with mass spectrometric detection”.

Scientific supervisor: Professor Dr. Dobrin A. Svinarov, MD, Ph.D, Dr. Med. Sc.

On the basis of order No. R-2051/18.07.2023 of the Rector of Medical University of Plovdiv prof. M. Murdjeva and the scientific jury decision, I have been engaged to prepare a statement about the dissertation work.

1. General presentation of the procedure and the PhD student

The presented set of materials is in accordance with Art. 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" at MU-Plovdiv; Regulations of MU-Plovdiv dated 28.01.2021 and includes all documents required for the procedure.

The doctoral student has attached 3 publications, all in refereed and foreign periodicals.

Dr. Tomov began his professional career in 2000 as a doctor in the emergency department, in 2004-2006 and 2013-2019 he was a resident doctor in the Clinical Laboratory, successively in the Medical Center of University Hospital "St. Georgi" - Plovdiv, Medical-diagnostic laboratory Chronolab Ltd, Medical-diagnostic laboratory "Sinevo" Bulgaria, Medical-diagnostic laboratory "Bodimed 99" Ltd. Dr. Tomov has acquired a specialty in Clinical Laboratory since 2012. From 2015 to the present, Dr. Tomov has been actively working in specialized laboratories with chromatographic mass spectrometric analysis, respectively, in the Technological Center for Emergency Medicine-Plovdiv and in the Department of "Bioorganic Chemistry" of the Medical University of Plovdiv.

Dr. Tomov has excellent theoretical and practical trainings in the field of biochemical, chromatographic and LC-MS analyses, which is evident from certification courses at leading foreign universities - Massachusetts Institute of Technology (MIT), Cambridge, US; Wageningen University & Research, Wageningen, NL; University of Tartu, EE.

Dr. Tomov is enrolled as a PhD student of independent training with order No. R-2750/02.11.2022. in the Department of "Medical Biology" on the topic of the dissertation work, passed all the required exams for the preparation of the PhD program and was dismissed with the right of defense by order No. R-2035/14.07.2023.

2. Relevance of the topic

The topic of the dissertation work is very actual. Currently, efforts are directed towards searching for suitable high-tech and highly specialized methods for evaluating the mechanisms of oxidative stress, damages and ways of their recovery. Free radicals and their derivatives cause

oxidative changes in lipids, proteins, nucleic acids and carbohydrates. The occurrence and progression of a large part of degenerative diseases is associated with the presence of oxidative stress, and the development and introduction of appropriate biomarkers provides an opportunity for their earlier diagnosis and proper treatment.

3. Understanding of the problem

It can be seen from the presented dissertation that Dr. Tomov knows the state of the problem in depth. It provides a detailed scientific literature review, very well structured and illustrated with graphs, in which it describes the progress made so far and poses still unsolved problems in this field. The published literary data regarding the preparation for conducting the research, the methods and the scientific conclusions have been examined in depth and critically.

4. Research methodology

Of the currently existing methods for the analysis of isoprostanes, the enzyme immunochemical and chromatographic methods are the most widely used. The number of liquid chromatographic methods published so far is very limited and most of them use highly sensitive analyzers such as QTRAP, QTOF or Fourier-transform infrared spectroscopy (FTIR). Dr. Tomov aims to develop and validate methods for the analysis of 8-isoPGF₂-alpha in various biological materials, in order to be proposed for diagnostics in daily practice.

5. Characteristics of the dissertation work and contributions

The dissertation work is very well structured and contains 109 pages - abbreviations used - 5 pages, literature review - 23 pages, aim and tasks - 1 page, materials and methods with a description of equipment, methods and clinical material - 16 pages, results and discussion - 47 pages, conclusions - 1 page, contributions - 1 page and bibliography 17 pages. The results are illustrated in 31 figures and summarized in 26 tables.

The presented protocols, results and conclusions are original, credible, informative and show a systematic and in-depth approach of the doctoral student. Dr. Tomov's efforts are aimed at developing and validating highly specialized methods with exceptional reliability and their medical application to patients in clinical practice.

The bibliography consists of 159 sources and mainly includes published results of leading researchers in the field of mass spectrometric research from the last 10 years.

The literature review is comprehensive, detailed, with a critical analysis by the author of the dissertation and with a presentation of the unsolved problems. A scientific explanation of the nature of free radicals, their importance and the mechanisms of damage caused by them at the cellular and molecular level has been reviewed. From the presented review, it can be seen that their influence cannot be limited to only one type of molecules, and often the peroxidation products of one type lead to damage of another type of molecules. It is believed that the products of the action of free radicals can be determined and measured, with which to assess the degree of oxidative stress. The published results on the levels of oxidative stress in patients with various chronic diseases, diabetes, autoimmune and oncological diseases and aging were analyzed.

The doctoral student makes a broad overview of the methods used in the scientific community to prove oxidative stress and determine its levels in various biological matrices - blood, urine, amniotic fluid, saliva, bronchoalveolar lavage or sputum. Direct analysis of free radical levels in the body is an expensive and time-consuming process requiring complex equipment, and is therefore not

widely available. Determining the products of the action of free radicals on biological molecules is an accessible method for proving the presence of oxidative stress in the body. Various classes of methods are reviewed in detail with their advantages and disadvantages, with the main emphasis on mass spectrometric methods

The purpose of the study is clear and specific, the 4 tasks set correspond to the set goal and lead to its implementation.

Materials and methods: The equipment and laboratory tools used, the principles of the analytical methods - liquid chromatographic method with mass spectrometric detection for the analysis of isoprostane 8-isoPGF2- α in blood plasma and saliva, as well as the clinical material are exhaustively described. The analytical conditions for carrying out the methods, the preparation of the biological material, the calibration and the quality assurance of the analyzes are specified.

The medical reliability of the analytical methods was determined in the study of patients with Hashimoto's autoimmune thyroiditis and patients with a metal-ceramic structure placed in the oral cavity. The research was approved by the Committee on Scientific Ethics at the MU-Plovdiv (protocol No. 4 /12.12.2019).

Appropriate and modern statistical methods were used to analyze the obtained results. Descriptive, parametric, non-parametric and correlation analysis methods were used.

The results, clearly, detailed and logically presented, are illustrated with appropriate tables and figures. Dr. Tomov presents textually and graphically the steps of method optimization, sample preparation for analysis, matrix effect analysis, etc. The methods were validated according to all the rules and criteria of the analytical reliability validation process.

The results from the application of the method for the analysis of 8-isoPGF2-alpha in blood plasma for the assessment of oxidative stress in patients with Hashimoto's thyroiditis are presented. Results showed the highest levels of oxidative stress in patients with hyperthyroid status. A direct correlation has been found between fat deposits in the body and higher levels of oxidative stress. As expected, the levels of 8-isoPGF2-alpha were higher in overweight patients than in normal or underweight patients.

When examining saliva, the difference in local oxidative stress before and after placement of a prosthetic construction in the oral cavity was sought. At the second hour after placement of the metal-ceramic construct, a significant decrease in 8-isoPGF2-alpha values was found compared to the initial levels, which coincided with the period of peak concentration of released metal ions determined by ICP-MS. A positive relationship was established between local oxidative stress and the application of CoCr alloys in the oral cavity.

The discussion is detailed, with understanding, with a comparison of the results with the data published in the literature from other scientific studies. Findings that differ from them or are established for the first time are noted. The importance of 8-isoPGF2alpha as a biomarker for free radical damage is highlighted.

At the end of the dissertation work, 4 conclusions are made, which are based on the obtained results and confirm the set goal.

The doctoral student has indicated 9 contributions of an original and confirmatory nature, which have promising scientific and practical application.

6. Publications and announcements of the dissertation study

In connection with the dissertation, 3 publications are presented, all in refereed national and international journals, which reflect and promote the results of the studies. Dr. Tomov is the first author in one of them, and second author in the other two. Two of the publications are in a journal with IF.

Dr. Tomov participated in 5 scientific forums at home and abroad, where he presented the results obtained to the scientific community.

Two intra-university scientific projects with the participation of the PhD student are presented, in which part of the research for the dissertation work was carried out.

7. Abstract

The abstract (43 pages) accurately reflects the dissertation work in summary.


CONCLUSION

The proposed dissertation work by Dr. Tomov reflects his personal involvement in the conducted research, which is very precise, labor-intensive and time-consuming, his strong commitment, desire and ambition to achieve qualitative and meaningful results. Dr. Tomov shows purposefulness in achieving his goals, critical thinking and the ability to interpret and generalize, which are exceptional indicators for evaluating his personal and professional qualities and skills for independent conduct of scientific research.

The dissertation work *contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet the requirements* of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of this Law and the Regulations of the Ministry of Education - Plovdiv. The presented materials and dissertation results **fully correspond** to the specific requirements adopted in connection with the Regulations of the Ministry of Education - Plovdiv for the application of the Law on the Development of the Academic Staff in the Republic of Bulgaria.

Based on above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I *propose to the honorable scientific jury to award the educational and scientific degree "Doctor" to Dr. Desislav Grozev Tomov* in a doctoral program in Medical Biology.

24.08.2023

Prepared by:  Българско научно общество
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