

STATEMENT OF OPINION



by **Victoria Sarafian, MD, PhD, DMSc,**

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Specialist in Clinical Immunology and Medical Biology,

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Regarding the thesis for the award of the educational and scientific degree "**PhD**":

Professional field - **Medicine**

Doctoral program - **Medical Biology**

**Author:** Dr. Desislav Grozev Tomov, PhD student on independent preparation

Topic: "**Determining Oxidative Stress Using Liquid Chromatography with Mass Spectrometric Detection**"

**Research Supervisor:**

Prof. Dobrin Svinarov, MD, PhD, DMSc

**General Information**

The review is prepared in accordance with Order No. R-2051/18.07.2023 of the Rector of the Medical University of Plovdiv, based on the submitted paper and electronic copy of the dissertation work. The overall impression from reading the dissertation is that it is conscientiously crafted and competently written. The doctoral candidate appears as a well-developed specialist in clinical laboratory with scientific knowledge in the field of medical biology. He has undoubtedly gained experience in their specialized training in mass spectrometry and have a scientific perspective in modern medical biology.

The dissertation is structured according to the rules for preparing a dissertation work, with an optimal balance between its individual parts. In its current form, the individual sections are presented as follows: introduction - 1 page; literature review - 24 pages; aim and objectives - 1 page; materials and methods - 15 pages; results and discussion - 32 pages; conclusions and contributions - 2 pages; and a total of 159 cited literary sources on 17 pages.

### **Relevance of the Topic:**

The high frequency, systemic manifestations, and disability caused by a large portion of degenerative, chronic inflammatory, and autoimmune diseases have brought them into the focus of scientific research. Oxidative stress plays a significant role in the complex etiopathogenesis of many of these conditions. Free radicals and their derivatives cause oxidative changes in lipids, proteins, nucleic acids, and carbohydrates. Some of these changes are reversible, but others act cytotoxically and can lead to cell death. Due to their extremely short existence time ( $10^{-9} - 10^{-12}$ sec), free radicals are currently primarily evaluated indirectly by measuring the products of their actions on various biomolecules.

The widespread adoption of metabolomics and proteomics in modern medical practice has contributed to the development of separation techniques and mass spectrometric analysis. Mass spectrometry guarantees high sensitivity and specificity, enabling quantitative assessment of substances based on their mass-to-charge ratio. It can also be used for the detection of isoprostanes, which are products of lipid peroxidation. Because they are stable molecules in all human tissues and organs, isoprostanes can be used to assess oxidative stress.

In the scientific literature, there is a limited number of published methods for their detection, probably due to the long and complex sample preparation process. Based on these data, the relevance of Dr. D. Tomov's dissertation work stands out, as he explores a highly promising research field. The scientific supervisor and the doctoral candidate argue for the necessity of developing and validating new methods for the analysis of 8-isoPGF<sub>2</sub>-alpha in various biological matrices, which could be proposed for diagnostic applications in daily practice. The final result of this extensive laboratory work demonstrates the potential of the researchers in creating a specific methodological protocol with broad clinical and laboratory applications.

### **Understanding of the Problem:**

The literature review demonstrates an excellent understanding of the problem and a creative analysis of the cited literature. It covers the characteristics, classification, and generation of reactive species in the body. The impact of free

radicals on proteins, nucleic acids, lipids, and carbohydrates is described in detail. Furthermore, the involvement of oxidative stress in the pathogenesis of neurodegenerative, metabolic, oncological, and autoimmune diseases is thoroughly presented.

A significant portion of the review is dedicated to presenting methods for analyzing oxidative stress. The author mainly focuses on methods for analyzing isoprostanes in a biological matrix and outlines the advantages and disadvantages of the techniques applied so far. Mass spectrometric detection is described in detail, showing a grasp of the details, not just the principles and mechanisms.

A good impression is made by the general conclusions of the literature review, which outline the unresolved problems and methodological challenges.

**The goal** is logically derived. It sounds clear and fully corresponds to the content of the work.

**The tasks** are formulated specifically and precisely. The doctoral candidate deserves credit for the exceptionally concise and concrete statement of the objectives, which demonstrates a mature and well-developed scientific approach. Many young doctoral candidates often get involved in numerous minor tasks and subtasks, losing focus. In this case, two methodological tasks are clearly defined, focusing on the development, optimization, and validation of a method for analyzing 8-isoPGF<sub>2</sub>-alpha in blood plasma and saliva. Following a logical sequence, the other two tasks are oriented towards the application of the developed methods for analyzing 8-isoPGF<sub>2</sub>-alpha in blood plasma as a biomarker of oxidative stress in patients with Hashimoto's autoimmune thyroiditis and in saliva as a local marker of oxidative stress in the oral cavity.

### **Materials and Methods**

The equipment and reagents used are accurately described, and the analytical methods are presented with impressive precision and accuracy, ensuring reproducibility of the obtained data.

The clinical material includes 95 patients with Hashimoto's autoimmune thyroiditis, divided into three groups based on the functional activity of the gland -

hypo-, eu-, and hyperthyroid status. The second clinical group consists of 35 patients with metal-ceramic dental restorations in the oral cavity. The participation of specialists in endocrinology and prosthetic dental doctors is appropriately reflected.

The financial support from the Medical University of Plovdiv through two intra-university projects is cited, which served as the basis for the development of the current dissertation work. This is evidence that the doctoral candidate and the research teams they collaborate with have significant scientific potential and experience in generating and implementing research projects.

### **Characterization and Evaluation of the Dissertation Work and Contribution**

**The results** are visually presented with 31 figures and 26 tables, showcasing the originality of the findings in line with the defined objectives of the dissertation.

The detailed description of the development and validation of the liquid chromatography with mass spectrometric detection method for determining 8-isoPGF2-alpha in blood plasma and saliva is provided. The method optimization, encompassing pre-analytical stages, sample preparation procedures, optimization of the chromatographic methods, and mass spectrometric detection, along with the final validation of the method, is systematically elaborated.

The analysis of the obtained results from applying the method to patients with Hashimoto's thyroiditis compared to the control group demonstrates the significance of 8-isoPGF2-alpha as a biomarker for free-radical damage in this condition due to its higher levels in patients compared to controls, despite the lack of statistically significant differences. Additionally, the study confirms the correlation between the degree of obesity, the presence of autoantibodies, and oxidative stress.

Regarding the fourth task, the results reveal a connection between the levels of isolated  $\text{Co}^{2+}$ - ions and the levels of 8-isoPGF2-alpha only at the second hour in stimulated saliva before and after placing a metal-ceramic restoration. Although no statistically significant differences in mean concentration levels are observed before and at the second hour after placing the metal-ceramic restoration, a trend towards increased isoprostane concentration in stimulated saliva is evident.

**The discussion** of the complex results appears to be relatively limited, likely due to the scarcity of scientific literature on the subject. This situation puts the author in a

position where they have to analyze their own data without relying on reliable scientific sources. On the other hand, this insufficiency in the discussion also highlights the original methodological approach and the achieved results. It would be beneficial to include future applications and directions that the doctoral candidate envisions for the implementation of the developed methods in the conclusion of the discussion.

**The conclusions** are clear, informative, and accurately reflect the essence of the research. I accept the conclusions from the dissertation as of the contents and personal achievement of the doctoral candidate. I appreciate the fact that they directly address the four specific tasks set out and present the research results in a convincing and systematic manner.

I fully acknowledge and accept the original **contributions** made in this dissertation, which are sufficient for a work of this nature. As for the confirming contributions, I do not believe it is necessary to specify them.

**The bibliography**, consisting of 159 literary sources, indicates the candidate's extensive knowledge of the field, and I trust that they are familiar with the full texts of these sources.

**The abstract** is prepared following standard guidelines and accurately reflects the content of the dissertation.

#### **Evaluation of Dr. Desislav Tomov's publications and personal contributions**

Dr. Desislav Tomov is the first author of three publications related to the topic of his dissertation. The two original articles were published in the *Journal of Medical Biochemistry*, with an IF of 2.157 and ranked as Q3 in SJR. The third publication appeared in *J Clin Exp Dent.*, ranked as Q2 in SJR.

The results of his scientific research have been presented at 2 national and 3 international forums. Dr. Tomov's personal contribution to the developed theme is evident from his participation in two successfully implemented scientific projects, funded by MU - Plovdiv.

Knowing Dr. Tomov's professional competencies as a clinical laboratory specialist, his additional qualification in the field of chromatographic analyses, and his

enhanced scientific capacity, I am convinced that the dissertation work is his own achievement under the skillful guidance of his scientific advisor - a proven expert in liquid chromatography with mass spectrometric detection.

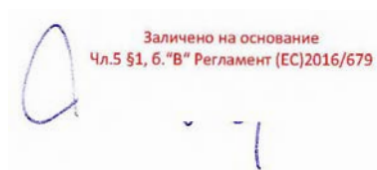
## CONCLUSION

The doctoral work of Dr. Desislav Grozev Tomov contains scientific and applied results that constitute an original contribution to the field of science and meet the requirements of the Law on Academic Staff Development in the Republic of Bulgaria for obtaining the educational and scientific degree of "Doctor" and the regulations of the Medical University of Plovdiv.

Based on the above, I confidently *give my positive assessment* of the conducted research and recommend to the members of the scientific jury to award the educational and scientific degree of "Doctor" in Medical Biology to Dr. Desislav Grozev Tomov.

24.07.2023

Reviewer:



Заличено на основание  
Чл.5 §1, 6. "В" Регламент (ЕС)2016/679

**Prof. Victoria Sarafian, MD, PhD, DMSc**