STATEMENT

by Assoc. Prof. Irena Ivanova Gencheva – Angelova, MD, PhD

Department of "Clinical Laboratory, Clinical Immunology and Allergology",

Medical University - Pleven

of a dissertation for awarding the educational and scientific degree "Doctor"

professional direction 7.1 Medicine

doctoral program 03.01.12. "Clinical Laboratory"

Author: Dr. Boyan Nikolaev Delev

Form of doctoral studies: independent training

Department: "Clinical Laboratory", FPh, MU-Plovdiv

Topic: "Study of thyroid function in healthy pregnant women from the Plovdiv region and development of population - specific reference limits of thyroid-stimulating hormone for each trimester of pregnancy"

Research supervisor: Prof. Tanya Deneva, MD, PhD, MU-Plovdiv

Assoc. Prof. Boyan Nonchev, MD, PhD, MU-Plovdiv

1. General presentation of the procedure and the doctoral student

The set of materials presented to me on paper and electronic media is in accordance with Art. 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv dated 28.01.2021 and includes the following documents:

– Application to the Rector of MU-Plovdiv for disclosure of the procedure for the defence of a dissertation work
– CV in European format with the doctoral student's signature
– a notarized copy of a higher education diploma
– orders for enrolment in doctoral studies, interruption of studies (due to maternity) and for continuation of studies; for deduction with right of defence
– an order for conducting an exam from the individual plan and a corresponding protocol for a passed exam or doctoral minimum in the specialty
– protocol from the departmental council for the preliminary discussion of the pre-dissertation work and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury
– dissertation work
– abstract
– a list of scientific publications on the topic of the dissertation
– copies of scientific publications
– list of participations in scientific forums
– list of noticed citations
– declaration of originality and authenticity of the attached documents
– other documents related to the course of the procedure

The doctoral student has attached 3 publications in connection with the dissertation.
The documents meet the requirements for the implementation of the dissertation defence procedure.

Biographical data of the doctoral student:

Dr. Boyan Delev was born on June 22, 1974. In 1992, he graduated "Vasil Levski" polytechnic high school in Yambol, and in 2002 Medicine at MU - Plovdiv. From 2003 to 2007 specializes and acquires a specialty in Clinical Laboratory. Since 2004, he has been working as a doctor in the department of Clinical Laboratory at UMHAT "St. Georgi" - Plovdiv, and since 2016 until now he is an assistant at MU-Plovdiv, Faculty of Pharmacy in the Department of Clinical Laboratory. From June 2021 he is a doctoral student of independent training at the Department of Clinical Laboratory, MU-Plovdiv in professional direction 7.1 Medicine.

2. Relevance of the topic

According to literature data, during pregnancy the reference range of thyroid-stimulating hormone (TSH) is lower compared to that in non-pregnant women, decreasing both the lower (by about 0.1 - 0.2 mIU/l) and the upper limit for indicator (by about 0.5 – 1.0 mIU/l).

Normal thyroid hormone concentrations are important for an uncomplicated pregnancy and optimal fetal growth and development. A large number of studies show that even mild hormonal dysfunction in this period is associated with an increased risk of complications and adverse consequences for the mother and the fetus. Thyroid hypofunction on one hand and gestational thyrotoxicosis on the other during pregnancy cause an increased incidence of reproductive disorders, spontaneous abortions, anaemia, gestational hypertension including intrauterine growth retardation, premature birth, low birth weight and neonatal respiratory distress.

The main diagnostic indicator in thyroid diseases is the measurement of serum thyroid-stimulating hormone and free thyroxine levels, and the optimal approach for interpreting the results is the development and use of population-based reference limits.

For the diagnosis of thyroid dysfunction during pregnancy, it is necessary to use different criteria than those for the general population, due to physiological processes and features affecting the thyroid status during this period. That is why the recommendations of authoritative endocrine organizations such as the American Thyroid Association and the European Thyroid Association state to prepare and apply trimester-specific values of indicators for each individual medical center, taking into account the characteristics of the local population.

Currently, there are no targeted and systematic studies on the problem in Bulgaria, and there are few laboratories that have established their own reference limits for TSH during pregnancy. The development of population-specific reference limits for TSH is a matter of priority importance for endocrinologists, and studies in this direction are encouraged in the consensus opinions of all European and world thyroid organizations.

All this defines the topic of the dissertation work as current, original and useful for clinical practice. The results are important for the development of science and practical behaviour.

3. Knowing the problem
It is clear from the extensive literature review that the doctoral student knows the state of the problem very well and presents a thorough analysis.

4. Research methodology
The nature of the study is a prospective study of a Bulgarian population of pregnant women from the Plovdiv region for each trimester of pregnancy. The women screened for participation in the study were recruited from outpatient practice, referred by the hypertension specialists and endocrinologists from the Plovdiv region and by the specialists from the Clinic for Endocrinology and Metabolic Diseases at UMHAT "St. Georgi" - Plovdiv. To determine reference limits, three groups of 120 pregnant women from each trimester of pregnancy over 18 years were selected. All participants were selected with the help of questionnaires previously developed for the purposes of the study, medical history, clinical examination by an endocrinologist, ultrasound evaluation of the thyroid gland and laboratory tests. Inclusion and exclusion criteria were in accordance with the generally accepted recommendations of the International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI). In all of them, biological material was collected and clinical laboratory analyzes were performed in the Clinical Laboratory of UMHAT "St. Georgi" - Plovdiv.

Survey, physical and instrumental methods, as well as clinical-laboratory studies, were used to collect scientific information. Software products - IBM SPSS 26 and REFVAL Program - were used for statistical processing of the obtained data.

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation work.

5. Characterization and evaluation of the dissertation work and contributions
The submitted dissertation covers a total of 114 standard typewritten pages illustrated with 14 tables, 11 histograms and 6 figures, and is organized into the following sections: Title page, Table of Contents (2 pages), Abbreviations used (2 pages), Introduction (2 pages), References overview (30 pages), Aim and objectives (1 page), Material and Methods (10 pages), Results (24 pages), Discussion (8 pages), Conclusions (1 page), Contributions (2 pages), Conclusion (1 page). The bibliography contains 263 literary sources, of which 5 are in Cyrillic and 258 are in Latin.

The structure and content are presented correctly and in detail. The most frequently used abbreviations are listed. The individual chapters and sub-chapters are properly designed, which gives clarity and clarity to the dissertation work.

The **literature review** is well structured and includes up-to-date information on the topic. Physiological features of thyroid function during pregnancy, as well as physiological dynamics of hormones in the mother and fetus, are presented in detail. The frequency of thyroid disorders during pregnancy and their manifestations are reviewed. The risks to pregnancy and the fetus due to thyroid dysfunction have been evaluated. The prevalence and impact of thyroid autoantibodies in pregnant women are reviewed. An assessment of thyroid function during pregnancy and a clinical-laboratory assessment of thyroid function in general were made.

The doctoral student knows the state of the problem perfectly, presents a thorough analysis and logically derives the thesis and the tasks of the dissertation work from the overview.

The aim of the thesis, namely to study the functional, morphological and immunological characteristics of the thyroid gland in healthy pregnant women and to develop population-specific reference intervals of TSH and fT4 for the different stages of pregnancy and related tasks are clear and precise formulated.

Results: 210 pregnant women were included in the study, 84 of them were observed during two trimesters, and 33 during three trimesters of pregnancy. The doctoral student derived demographic and clinical data for each study group. Thyroid stimulating hormone (TSH), free thyroxine (FT4), anti TPO- and anti (Tg) antibodies, free triiodothyronine (fT3) were investigated. The statistical significance of the relationship between weight, height and age for the respective trimester and laboratory parameters of the thyroid gland for the different trimesters of pregnancy was assessed with non-parametric analysis. Family history of thyroid pathology, pregnancy sequence, infertility, miscarriages, and smoking were also taken into account. TSH and fT4 levels were determined in healthy pregnant women with negative thyroid antibody titres in the first, second and third trimesters of pregnancy. The levels of TSH and fT4, and anti-TPO and anti-Tg in the selected group of pregnant women in the 1st, 2nd and 3rd trimesters of pregnancy were determined and compared. The functional thyroid indicators of healthy pregnant women were analyzed depending on the morphological characteristics of the thyroid gland, assessed by the thyroid volumes of the gland and the physiological changes in thyroid function in the individual trimesters of pregnancy.

Population-specific reference intervals for TSH and fT4 were determined for the I, II and III trimesters of pregnancy, in healthy pregnant women from the studied population. The limits of the population-specific reference area were determined according to the method of statistical processing of the reference values recommended by the International Federation of Clinical Chemistry and the Institute of Clinical and Laboratory Standards, and the recommendations of these organizations regarding the selection of pregnant women were followed. The type of statistical distribution of the concentration values of the measured indicators for the first, second and third trimesters of pregnancy was investigated. For this purpose, an assessment was made based on histograms, Kolmogorov-Smirnoff test, coefficient of asymmetry (skewness) and kurtosis (kurtosis), P-value.

The doctoral student's own **research** is presented consistently, described comprehensively and illustrated with a sufficient number of tables and figures.

In the **"Discussion"** section, the dissertation student makes a thorough analysis of the obtained results. Evaluates in detail the comparative analysis and the statistical significance of the obtained results. Compares the data from their population-specific reference intervals for TSH and fT4 for the I, II and III trimesters of pregnancy with the results of other authors.

In the dissertation, 6 **conclusions** are summarized, which are in accordance with the obtained results and are logically deduced from the set tasks.

The dissertation student has defined a total of **6 contributions** of a scientific and applied nature.

6. Evaluation of the publications and personal contribution of the doctoral student

The list of scientific works related to the dissertation includes 3 articles in scientific journals, one of which in a journal referenced in Scopus and/or Web of Science. In all three publications, Dr. Delev is the first author. The dissertation student has 2 participations in scientific forums and 1 participation in a scientific project of MU-Plovdiv.

7. Abstract

The abstract has a total volume of 48 pages, in terms of content and quality, it fully presents the results of the scientific research in the dissertation.

CONCLUSION

Dr. Delev's dissertation contains scientific and applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB 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 LDASRB.

The dissertation shows that the doctoral student, Dr. Boyan Delev, has in-depth theoretical knowledge and professional skills in the scientific specialty Clinical Laboratory, demonstrating qualities and skills for independent conduct of scientific research.

Due to the 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 honourable scientific jury to award the educational and scientific degree "Doctor" to Dr. Boyan Nikolaev Delev in a doctoral program in Clinical Laboratory.

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

20.02.2024 Prepared the opinion: ....

(Assoc. Prof. Irena Gencheva, MD, PhD)