

REVIEW

By: Associate Professor Dr. Milena Krasimirova Nikolova-Vlahova, MD, PhD

Clinic of Nephrology, University Hospital "St. Iv. Rilski", Department of Internal Medicine, MF, MU-Sofia, Specialties: Nephrology and Internal Medicine

Concerning: Dissertation (PhD thesis) for the educational and scientific degree "Doctor" (PhD), Dr. STANISLAVA LIUBOMIROVA ILIEVA, on the topic: "ROLE OF SOME PATHOGENETIC AND GENETIC FACTORS IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES"

Scientific mentor: Assoc. Prof. Dr. Lachezar Boyanov Lozanov, MD, PhD

Scientific field: 7.1 Medicine

Doctoral program: Internal Medicine

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Brief biographical data of the dissertation candidate

Dr. Stanislava Ilieva was born in Sofia, graduated from the 7th secondary school "St. Sedmochislenitsi" with excellent results, and in 1997 she graduated from the Faculty of Medicine of the Medical University of Sofia, specialty of Medicine. She has two clinical specialties - Internal Medicine and Nephrology, and numerous publications in these fields, both in Bulgarian and English. She has professional qualifications in conventional abdominal ultrasound and biopsy methods in nephrology. For more than 25 years she has worked in the field of internal medicine and nephrology, successively in the Nephrology Clinic at the University Hospital "Ts. Ioanna ISUL" and in the Nephrology Department at the Clinic of Internal Medicine, „Acibadem City Clinic UMBAL Tokuda Hospital" Ltd., Sofia. She is fluent in written and spoken English and Russian.

1. Topic of the dissertation: relevance, significance, appropriateness and knowledge of the problem

The topic of the present dissertation, "ROLE OF SOME PATHOGENETIC AND GENETIC FACTORS IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES", is particularly relevant, since it explores the importance of genetic factors and some pathogenetic mechanisms in the development of diabetic nephropathy - an increasingly common complication of type 2 diabetes mellitus, which has become one of the "epidemics of the 21st century". Dr. Ilieva has serious and in-depth research in this direction, which is a significant prerequisite for the development of the present dissertation.

2. Methodology of the dissertation

In the development of this dissertation, Dr. Ilieva used modern and appropriate research and statistical approaches for collecting and processing data, with high reliability of the results.

3. Characteristics of the dissertation

The dissertation work is presented on 103 standard printed pages, illustrated with 15 figures and 33 tables. The bibliography includes 148 literary sources, including in Bulgarian, most of which are from the last 10 years.

The literature review is written in correct and easily readable literary Bulgarian, with adequate scientific terminology without unnecessary use of loanwords and foreign words, and presents in detail and in depth the current knowledge about the pathogenesis of diabetic nephropathy and the importance of various, including genetically determined, factors for the development of pathohistological changes, and the newer possibilities for an individualized diagnostic and therapeutic approach are also presented.

The aim of the dissertation is formulated precisely and clearly: to establish a relationship between specific clinical and laboratory biomarkers and the manifestation of diabetic nephropathy in patients with type 2 diabetes mellitus, with a view to identifying predictors for its development and optimizing the diagnosis of diabetic kidney damage.

To achieve this aim, the PhD student has formulated six clear tasks:

1. To determine the histological diagnosis after performing kidney biopsy on patients with CKD and type 2 diabetes and to analyze the frequency of histological findings in the observed groups.
2. To analyze certain clinical and laboratory indicators in biopsied diabetics and compare the results obtained with a control group of patients with histologically proven nephropathies, without diabetes.
3. To determine the possible relationship between the presence of diabetic nephropathy and the remaining micro- and macroangiopathic complications of diabetes.
4. To establish the existence of a relationship between certain biomarkers (clinical, laboratory, genetic) in diabetics with and without DN, as well as between all diabetics and non-diabetics.
5. To establish specific predictors of diabetic kidney complications in patients with type 2 diabetes.
6. To propose a diagnostic approach and recommendations for clinical practice in order to optimize the diagnosis of diabetic kidney complications.

Materials and methods.

The study was conducted on 91 patients hospitalized in the Department of Nephrology, Clinic of Internal Medicine of "ACC UMBAL Tokuda" Hospital Ltd. for the period 01.2018 - 02.2024. All patients had CKD and histologically proven nephropathies. 55 of the patients had type 2 diabetes mellitus, established before or during the diagnosis of kidney disease, and the remaining 36 were without diabetes. The patients were aged between 22 and 76 years, 55 (60.4%) of them were men and 36 (39.6%) were women. In almost all patients, percutaneous puncture renal biopsies were performed in the Department of Nephrology of "ACC UMBAL Tokuda" Hospital Ltd. in the above-mentioned period or before. The study did not include patients with type 1 diabetes, corticosteroid-induced diabetes, or those in whom diabetes occurred after the diagnosis of kidney disease.

The patients were divided into 3 groups, depending on the presence of diabetes and diabetic nephropathy:

- Group I: Patients with type 2 diabetes and histologically proven diabetic nephropathy.

- Group II: Patients with type 2 diabetes in whom other, non-diabetic kidney damage is present in the renal histology.

- Group III (control) Patients with histologically proven nephropathies, without diabetes. The indications for performing a puncture renal biopsy in all patients are: the presence of increased albuminuria or proteinuria, measured in 24-hour diuresis and/or impaired renal function.

The following clinical and laboratory parameters were evaluated in the three groups:

- General assessment of clinical status by organs and systems
- Presence of arterial hypertension
- Presence of complications of diabetes:
 - o Diabetic retinopathy
 - o Diabetic polyneuropathy
 - o Diabetic macroangiopathy
 - o Diabetic gangrene
- Presence of obesity, determined by measuring Body Mass Index (BMI)
- Laboratory indicators of renal function:
 - o Cystatin C
 - o Serum creatinine
 - o Estimated glomerular filtration rate (e-GFR)
- Laboratory indicators of metabolic control: o Glycated hemoglobin o Lipid profile, including:
 - * Low-density lipoproteins (LDL)
 - * High-density lipoproteins (HDL)
 - * Triglycerides
 - * Uric acid
- Excretion of total protein and albumin in 24-hour diuresis
- Laboratory markers of inflammation and vascular damage in the blood:
 - o C-reactive protein (CRP)
 - o Interleukin 6 (IL-6)
 - o D-dimer
 - o Fibrinogen
- Polymorphism study of the methylenetetrahydrofolate reductase gene (MTHFR) gene, in particular the variants A1289C and C677T.
- Serum homocysteine and folic acid levels

- Thyroid function tests:

- o Thyroid-stimulating hormone (TSH)
- o Free thyroxine (FT4)
- o Microsomal antibodies (MAT)
- o Thyroglobulin antibodies (TAT).

Thus, using standard methods, all subjects were assessed for the presence of obesity and arterial hypertension, the presence of micro- and macrovascular complications of diabetes mellitus, standard blood and urine clinical and laboratory tests were performed, including some newer markers (e.g., cystatin C), as well as histological evaluation of material from a puncture kidney biopsy, in the collection of which the doctoral candidate actively participated. Standard indicators of metabolic control and newer indicators of vascular damage and inflammation (CRP, IL6, d-dimer, coagulation indicators, including fibrinogen) were studied; homocysteine and folate in serum, along with MTHFR polymorphism, as well as thyroid function and anti-thyroid antibodies.

The statistical analysis was performed using standard and modern methods.

Results

The results of the biopsy (renal core biopsy) studies indicate that patients with type 2 diabetes may develop other non-diabetic kidney damage, including hypertensive nephropathy and tubulointerstitial changes, and idiopathic glomerulonephritis. In addition, in patients with diabetic nephropathy, a predominance of male gender and an older age of patients is reported compared to non-diabetics. Diabetics also have a higher incidence of arterial hypertension, higher levels of body mass index and obesity. Diabetics with proven diabetic nephropathy have a higher incidence of retinopathy, but not of other vascular complications.

The metabolic control is worse in diabetics, including lower levels of HDL.

Patients with diabetic nephropathy have higher microalbuminuria and higher proteinuria, higher levels of inflammatory and pro-coagulant markers, a tendency towards higher levels of homocysteine and, respectively, lower folate. Thyroid antibodies and dysfunction are demonstrated slightly more frequently in non-diabetics, and in diabetics there are no clinically significant differences in the frequency of autoimmune thyroid disease compared to non-diabetics. However, the presence of some type of deviation in thyroid function or ADT is present in 16% of diabetics without DN, which provokes an active search for thyroid diseases in all diabetics.

The discussion presents in an accessible way the PhD student's own reasoning on the obtained results and compares them with the data from the studies conducted so far by other authors.

There are 9 conclusions and they correspond to the set goals and objectives.

Three recommendations for clinical practice are clearly stated:

- Active monitoring for the presence of CKD in patients with type 2 diabetes and over 60 years of age, with the presence of accompanying diabetic retinopathy, low levels of HDL in serum and elevated triglycerides.

- One should not forget that there is a high risk of CKD in patients with type 2 diabetes and elevated levels of D-dimer, IL-6 and fibrinogen in the blood, in the absence of an inflammatory, vascular-related cause or coagulation disorders.
- Performing renal biopsy in all diabetics with impaired renal function and/or the presence of varying degrees of proteinuria, when there are no contraindications.

A total of 7 contributions are stated – both theoretical and scientific-practical, which fully describe the merits of this dissertation work.

The bibliography encompasses 148 references, both in Bulgarian and in English.

4. Publications and personal contribution of the dissertation candidate

As related to the present dissertation work, Dr. Ilieva has presented the required number publications in scientific journals and a report at an international scientific forum, which meet the requirements for acquiring the scientific and educational degree "Doctor".

5. Abstract

The presented Abstract is structured correctly, in accordance with the requirements, and clearly presents the results obtained and the related figures and tables, and their discussion.

The gratitude expressed by Dr. Ilieva to her colleagues, collaborators and to her family in the Acknowledgements section makes a particularly good impression.

The small number of spelling errors in no way belittle or undermine the merits of the dissertation work.

I have no substantive comments in relation to the previous dissertation work and the abstract to it.

In conclusion, Dr. Ilieva has presented a dissertation for the acquisition of the scientific and educational degree "Doctor" on an up-to-date topic in the field of internal medicine. The dissertation is written in good literary Bulgarian, structured correctly, with well-chosen methods, a clear goal and specific tasks, and clearly illustrated results.

The presented dissertation meets all the requirements of the relevant laws in the Republic of Bulgaria and the Regulations for their implementation, as well as the Regulations "Acibadem City Clinic UMBAL Tokuda" Hospital Ltd., for the acquisition of the educational and scientific degree "Doctor" (PhD).

Based on everything stated so far, I confidently give my vote "FOR" the awarding of Dr. Ilieva with Doctor's degree (PhD) and I would like to recommend to the other reviewers and the members of the scientific jury to vote positively.

November, 2024

Sincerely:

Sofia

Assoc. Prof. Dr. Milena Krasimirova Nikolova-Vlahova, MD, PhD