To the Chairman of the Scientific Jury

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of the Rector of Medical University Varna and

according Protocol No.1/ № 1/Dec 20, 2021

REVIEW

by assoc. prof. Mira Valentinova Siderova, MD, PhD

scientific specialty – Endocrinology

associate professor at the Department of Endocrinology, Second Department of Internal Medicine, Medical University of Varna

of dissertation

of Dr. Radina Stoyanova Dimitrova, PhD student in full-time education, on the topic

"Comparative characteristics of metabolic markers in assessment of postmenopausal bone health" for awarding of educational and scientific degree "PhD" in professional field 7.1. Medicine, doctoral program "Endocrinology"

with scientific supervisor: Prof. Dr. Kiril Hristov Hristozov, PhD

1. General presentation of the procedure

The presented set of materials corresponds to the requirements of the procedure for acquiring educational and scientific degree "PhD" according to the Regulations of Medical University of Varna and includes all necessary documents.

2. Brief biographical data of the applicant

Dr. Radina Dimitrova was born in 1985 in the town of Shumen. She graduated with honors from the High School of Natural Sciences and Mathematics in 2004 and medicine at Medical University of Varna in 2010. Until 2013, Dr. Dimitrova worked in the Emergency Department of the University Hospital "St. Marina "-Varna, and in 2014 she joined the team of the Clinic of Endocrinology and Metabolic Diseases. In 2016 she was appointed assistant at MU-Varna at the Department of Internal Medicine, Unit of Endocrinology. Dr. Dimitrova acquired a specialty degree in Endocrinology and metabolic diseases. Since 2018 she is a PhD student at the Second Department of Internal Medicine, Endocrinology, MU-Varna. Dr. Dimitrova is certified in the

methods of cervical ultrasound, fine-needle biopsy of the thyroid gland, parathyroid lesions and cervical lymph nodes.

3. Relevance of the topic

Osteoporosis and metabolic syndrome are socially significant health problems worldwide. Osteoporosis is the most common metabolic bone disease, usually asymptomatic before fractures occur. The latter represent a significant burden for both the patient and society, as they are associated with high morbidity, mortality and high healthcare costs. Metabolic syndrome, in turn, is a set of risk factors that predispose to the development of type 2 diabetes and cardiovascular diseases. What at first glance unites the two pathologies - osteoporosis and metabolic syndrome is their high frequency, which increases with age. Little is known about the relationship between these two conditions, which is the subject of scientific research of the dissertation of Dr. Radina Dimitrova. The novelty of the problem is underlined by the expected increase in life expectancy and the proportion of elderly people with osteoporosis and metabolic syndrome. The ambiguous results in previous studies on the relationship between osteoporosis and metabolic disorders, as well as limited data in Bulgaria, determine the relevance of the chosen topic.

4. Evaluation of the dissertation

The dissertation is written on 174 pages, illustrated with 22 tables and 84 figures and is well formed in the following main sections: Title page (1 page), Content (2 pages), Abbreviations (2 pages), Introduction (2 pages) , Literature review (40 pages), Purpose, tasks and hypotheses (1 page), Material and methods (4 pages), Results and discussion (90 pages), Conclusions, contributions and conclusion (6 pages), References (25 pages). The bibliography contains 411 titles, of which 3 in Bulgarian and 408 in English language.

5. Structure of the dissertation

The literature review is very well structured and includes up-to-date information on the epidemiology, etiology and pathogenesis of osteoporosis and metabolic syndrome. The data on the relationship between metabolic syndrome and bone health have been reviewed in detail. The doctoral applicant presents an in-depth analysis of the relationships: bone and visceral adipose tissue, bone and insulin resistance, as well as bone and lipids, bone and arterial hypertension, including the impact of different classes of antihypertensive drugs on bone health. Dr Dimitrova is well aware of the state of the problem and has presented a creative assessment of the analyzed literature.

The aim of the dissertation is clearly stated, namely to establish the relationship between bone health and metabolic profile in postmenopausal women by comparing certain metabolic parameters and inflammatory markers and to assess the relationship of metabolic syndrome with bone integrity. To achieve this goal, 6 specific **tasks** have been set, correctly selected and precisely formulated (finding tasks 4, 5 and 6 especially interesting):

1. Evaluation of bone health in postmenopausal women by DEXA BMD measurement at the level of lumbar spine and proximal femur, assessment of bone metabolic markers and calculation of fracture risk.

2. Assessment and analysis of the relationship between age and duration of menopause with bone health.

3. Evaluation and analysis of the relationship of some anthropometric, hormonal, metabolic and inflammatory parameters with bone health in postmenopausal age.

4. Evaluation and analysis of the relationship between blood pressure and the intake of antihypertensive drugs with bone health in postmenopausal age.

5. Assessment and analysis of the complex relationship of metabolic syndrome with bone health in postmenopausal age.

6. Determination of risk profile for compromised bone health in postmenopausal age.

Participants and methods:

For the purposes of this dissertation, Dr. Dimitrova selects 84 postmenopausal women above 45 years of age from Northeastern Bulgaria for the period 2019-2020. The inclusion and exclusion criteria are adequate to the purpose and design of the study. Clinical, laboratory and X-ray examination methods are optimally selected. Adequate statistical analysis - analysis of variance (ANOVA) was used; variation and correlation analysis, as well as regression analysis - univariate and multifactor linear stepwise analysis for processing large amounts of data.

Results and discussion:

The results of the study are presented correctly in 9 sections, well illustrated in tables and graphical versions, accompanied by adequate and objective commentary.

The first section presents the results of the bone health assessment of the participants by performing DEXA osteodensitometry, examining markers of bone metabolism and calculating fracture risk. 46.6% of the surveyed women have osteopenia, 35.7% have osteoporosis and 17.9% have normal bone and mineral density (BMD), respectively, the latter accepted as healthy controls. The data are presented in detail, including tables with clinical and laboratory data and a comparative analysis between the three groups is performed. The higher levels of bone markers found at lower BMD confirm the hypothesis that postmenopausal osteoporosis is associated with increased bone turnover.To distinguish the clinical group of patients with osteoporosis from healthy controls, the doctoral student determined cut-off values of osteoclacin (5.18 ng / ml (AUC = 0.621 (0.492–0.749), p = 0.006), Beta Crosslaps (0.44 ng / ml (AUC = 0.510) 0.343–0.678), p = 0.008) and the DPD/Cr ratio (5.67 nmol / mmol (AUC = 0.510 (0.355–0.666), p = 0.007), and calculated the diagnostic accuracy of these markers separately

and in combinations. A positive association between serum ionized calcium and BMD was also established. Additional analysis of the females on thiazide diuretic treatment showed a strong positive relationship between calcium retention in the body and BMD ($r = 0.381$; $p = 0.05$) in the studied skeletal areas and it was assumed that calcium retention through the kidneys has a beneficial effect on bone mass in patients taking thiazide. The analysis of vitamin D levels shows that 78.6% of the surveyed women have a level of 25 (OH) D below the desired values. A positive correlation between the level of 25 (OH) D and BMD was found only in healthy controls, supporting the role of this vitamin in maintaining normal bone mass, as well as the importance of therapeutic correction of low levels vitamin D for the prevention of osteoporosis.

The second section examines the relationship between the age and menopause duration and bone health. As expected, the women with proven osteoporosis in the observed cohort are older and have a longer history of menopause, both compared to healthy controls and compared to women with osteopenia. Important conclusion of interest is that 70% of the changes in bone density are due to the long duration of menopause and the advanced age of women. The age cut-off above which the risk of osteoporosis increases is calculated to be 62.5 years with a sensitivity of 56.7% and a specificity of 70.4%. The cut-off value of menopausal duration above which the risk of osteoporosis increases is set at 12.5 years with a sensitivity of 63.3% and a specificity of 72.2%.

The third section is devoted to the analysis of anthropometric parameters and their relationship to bone health. After the initially established positive correlations of waist circumference and body mass index (BMI) with BMD, as well as negative associations on regression analysis of the two anthropometric indicators with fracture risks, it was assumed that abdominal obesity and higher body weight, but not extreme obesity, could be positively associated with postmenopausal bone health.

The fourth section presents the results of an assessment of the relationship between carbohydrate metabolism and bone health. With fasting blood glucose (BG) ≥ 5.6 mmol/l are 51% of the total cohort with the highest percentage in the group of osteopenia (56%), and with BG at 120 min ≥ 7.8 mmol/l (OGTT) are 13% of individuals distributed among cases of osteopenia and osteoporosis. With HOMA index> 2 are 46% of the observed sample, with the highest share in healthy controls (60%). The correlations between BMD and insulin level and the changes in these dependencies in insulin resistance (HOMA index> 2) are well graphically presented. It has been suggested that the osteoanabolic effect of insulin depends on the persistence of insulin sensitivity. Individuals with a BG <5.6 mmol / l were found to have higher levels of the bone formation marker (BC). Dependencies of fracture risk with the level of BG, as well as the level of vitamin D and BG and insulin (both fasting and stimulated at 120 minute from OGTT) are also presented. The level of 25 (OH) D is negatively related to the level of insulin at 120 minutes and this relationship is maintained after the inclusion of anthropometric parameters.

The fifth section analyzes the relationship between lipid metabolism and bone health. Although the differences in the levels of lipid parameters between the groups analyzed according to the BMI did not reach statistical significance, it was found that in women with osteoporosis the percentage of people with total cholesterol levels above the upper reference limit is higher (77%) than in healthy controls (47%) and cases of osteopenia (64%). Significantly lower levels of Beta Crosslaps and DPD/Cr are found in women with osteoporosis with total cholesterol levels> 5.18 mmol/l. Higher triglyceride levels in the present study were associated with higher lumbar spine BMD, especially in women with osteopenia. Data on LDL-cholesterol and bone integrity do not suggest a significant association between this lipid index and postmenopausal osteoporosis.

The sixth section discusses the results of the study of inflammatory markers (TNFα, CRP) and bone health. Mean TNFα values increase with decreasing BMD. The highest share of women with TNFα> 8pg/ml is in the group of women with osteoporosis (33%). A negative relationship has been found between the levels of TNFα and BMD in the lumbar vertebrae, but it loses its relevance in terms of body weight, which is positively associated with BMD.

The seventh section presents interesting data on blood pressure and the role of antihypertensive therapy for bone integrity. Arterial hypertension was diagnosed in 69% of the participants, of which 8% were newly diagnosed. The lowest BMD at the level of lumbar vertebrae and thighs is reported in women with stage III hypertension, which is interpreted by the doctoral student as a result of a higher age in this group as well as possible bone adverse effects in terms of targeted organ damage due to hypertension. Of interest is the highest BMD in the group of women with stage II hypertension. Dr. Dimitrova suggests a protective bone effect of some antihypertensive drugs, as normotensive women and those with newly diagnosed hypertension (stage I) do not take such medications and have a lower BMD.

A detailed analysis of the intake of ACEi / ARB, thiazides, beta-blockers, calcium antagonists and, respectively, BMD by areas and markers of bone metabolism according to the intake of antihypertensives was performed. No significant difference was found between the levels of bone markers according to the presence of arterial hypertension and its stage. However, this is reported after considering the use of antihypertensive drugs in women with osteoporosis. Better parameters of BMD at the level of the femoral neck and proximal femur were found when taking ACEi or ARB in the group of healthy controls. In the group of patients with osteopenia, the intake of these drugs is associated with higher BMD in both the thigh and lumbar spine. However, no positive effect was observed in the group of patients with osteoporosis. Similar results were observed with thiazide diuretics. On the other hand, the intake of beta-blockers is positively associated only with lumbar BMD in healthy controls. Calcium antagonist intake has been positively associated with BMD at both lumbar and proximal femur levels in healthy controls and in patients with osteopenia. In patients with osteoporosis, better BMD parameters are observed only in the area of the femoral neck and proximal femur. The hypothesis of the potential pleiotropic effect of antihypertensive drugs on bone, shared by other authors, is presented in the discussion. The better BMD rates found in different skeletal

regions in the healthy controls group and among women with osteopenia taking ACEi / ARB, thiazide diuretics, beta-blockers and/or calcium antagonists raises the question of the importance of these antihypertensive medications in prevention of osteoporosis with concomitant hypertension. On the other hand, the lack of such data in the group of women with osteoporosis taking ACEi/ARB and/or thiazide diuretics suggests a loss of this protective effect in osteoporosis or negative impact of additional factors such as older age, longer duration of MP or comorbidities.

The eighth section is devoted to the relationship between bone health and metabolic syndrome (MetS), which was found in 48.8% of women surveyed. The results of the comparative analysis of T-score and BMD according to the presence and absence of MetS showed a tendency towards higher bone density in women with MetS. The multifactor analysis conducted by Dr. Dimitrova shows that the reported positive relationship between waist circumference and bone mass is determined by higher body weight and probably greater mechanical load on the skeleton. Similarly, body weight and BMI, but not waist circumference, correlate negatively with fracture risk. Fasting blood glucose and glucose at 120 minute of OGTT are negatively associated with bone integrity. Regarding the levels of markers of bone metabolism, no significant differences were found between individuals with and without MetS in the general cohort. In women with osteoporosis and MetS compared to these with osteoporosis without MetS, significantly lower values of osteocalcin ($p < 0.01$) and Beta Crosslaps ($p < 0.05$) were found.

The last section examines the risk profile for deteriorating bone health in postmenopausal age. Leading risk factors for osteoporosis are longer duration of menopause and older age. An additional risk factor is the lower body weight with a cut-off value of 66.5 kg, below which the BMD decreases and the fracture risk increases. Lower basal insulin levels and higher stimulated insulin (120 minute of OGTT) are also associated with an increased fracture risk. According to the identified additional risk factors, the doctoral student offers a complex diagnostic and therapeutic algorithm for postmenopausal women.

The ten **conclusions** are in accordance with the obtained results and follow the tasks set in the dissertation.

The **contributions** of the dissertation are divided into three categories: scientific-theoretical (2), scientific-practical (4) and confirmatory (3). The most important are the following contributions:

- Connection of the metabolic syndrome and its individual components with BMD, markers for bone metabolism and fracture risk in postmenopausal women is studied for first time in Bulgaria.

- For the first time in Bulgaria, relationship of blood pressure with bone health in postmenopausal age is assessed, as well as the associations of bone parameters with hypertension stage and the intake of antihypertensive drugs.

- A direct comparison was performed between the serum marker for bone resorption Beta Crosslaps and the urinary marker for bone resorption pyrilinx D/creatinine in urine. The performed analyzes emphasize the greater diagnostic value of the serum marker Beta Crosslaps and draw attention to extraosseous influences on the levels of the pyrilinx D/creatinine ratio in the urine.

- Based on the results obtained, a risk profile for compromised bone health in postmenopausal age was determined.

6. Abstract and publications related to the dissertation

The abstract is completely sufficient in content and quality to present the main results achieved in the dissertation. The doctoral student has attached to the documentation 5 full-text publications (4 reviews and 1 with results) related to the dissertation. The attached list meets the requirements needed for educational and scientific degree "PhD".

7. Critical remarks and recommendations.

I do not have critical remarks and recommendations to the conducted research and the materials provided to me.

In conclusion, the dissertation I reviewed, dedicated to a up to date and socially significant topic, reflects an in-depth and valuable work, impressive with its precise statistical processing. I believe that the study meets the requirements of the Academic Staff Development Act in Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Development of Academic Staff at Medical University of Varna for obtaining the scientific and educational degree "PhD" and I vote **positively** for awarding the degree "PhD" to Dr. Radina Stoyanova Dimitrova.

Feb 07, 2022

Varna

Reviewer:

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