To the Chairman of the Scientific Jury,
Assigned by Order
№ P-109-585/ 17.12.2021
of the Rector of Medical University – Varna

Review

By Assoc. Prof. Alexander Dimitrov Shinkov, MD, PhD
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On the Doctoral Thesis titled: Comparative characteristics of metabolic markers in assessment of postmenopausal bone health
Authored by the candidate Radina Stoyanova Dimitrova MD, a doctoral fellow at the Department of Endocrinology, Medical University – Varna.
Area of higher education: code 7, Healthcare and Sport, professional trend code: 7.1 Medicine, Scientific specialty: Endocrinology

I disclose no co-authored scientific publications or any conflicts of interest with the candidate as required by Law on the Development of the Academic Staff in the Republic of Bulgaria.

The presented documents comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

Brief highlights from the biography of dr Radina Dimitrova

Dr Radina Dimitrova has graduated the Medical University- Varna in 2010. In 2018 she completed her specialty training in endocrinology and metabolism. Since 2016 she has been teaching as an assistant professor at the Second Department of Internal Medicine at the Medical University – Varna. She has since acquired significant experience in the diagnostics and treatment of endocrine disorders and has evolved into a young scientist with a wide scope of interests. Dr Dimitrova has participated in a number of local and international training courses and scientific meetings.

Relevance of the topic and feasibility of the aims of the dissertation

The increase in life expectancy, the changes in lifestyle with high caloric nutrition and low physical activity have increased recently the incidence of several disorders like obesity, diabetes, arterial hypertension and osteoporosis. The osteoporosis observed after the menopause and with ageing is associated with fractures which raise the morbidity and mortality. Therefore the individual assessment of the risk for osteoporosis and fractures is crucial for the early prevention and treatment of the high-risk individuals. The metabolic disorders are risk factors for cardiovascular morbidity and mortality and their prevalence rises with ageing as well. An association between bone health and the metabolic disorders has been described – higher bone mineral density (BMD) in some postmenopausal women with increased body weight that might be explained by the increased aromatase activity, the preserved BMD and increased fracture risk in type 2 diabetes mellitus, the possible role of osteocalcin as a molecule linking bone and intermediate metabolism etc.

Hence further research in the field would be valuable both from scientific and theoretical and from clinical points of view.

Structure of the dissertation

The manuscript is structured according to the common standards applying to a PhD thesis. It is printed on 174 pages including 411 references, of which 6 are published by Bulgarian authors. The data are visualized in 22 tables and 84 figures. The Review of the literature comprises 40 pages, the Objective and the tasks – one page, the Material and methods section – four, the Results and discussion – 90 and the Conclusions, Scientific contributions, Summary and List of publications – 7 pages.

The thesis is written in good Bulgarian.

The review of the literature demonstrates good knowledge of the matter. It is structured clearly and is well focused on the topic of the thesis, permitting a logical formulation of the objectives and the tasks of the dissertation.

The formulation of the objective is concise and clear. The tasks are specific, well-defined and reasonable.

The design of the study is cross-sectional. The participants are divided into three groups according to the WHO-defined T-score categories. A huge number of laboratory tests and other measurements were done followed by in impressive variety of analyses of the data. The applied methods have been chosen reasonably and in agreement with the planned tasks.

The results follow the structure of the literature review. Some of the more important and interesting observations include the confirmation of the association between body size and BMD. In my opinion it might be interesting to include in the analysis the vertebral body areas which could show whether geometry or rather the microarchitecture mediate that association. Other key results include the lack of association between the serum lipid levels and bone metabolism, the inverse relationship between the vitamin D levels and the body weight and the high prevalence of vitamin D deficiency among the subjects with osteoporosis. The measurement of osteocalcin deserves a special attention as well. Further on one can assume from the presented data that some analyses might yield statistically significant results and permit firmer conclusions if the sample vas larger. One such example is the difference in the osteocalcin levels among the groups with different body mass index.

The author has presented five publications related to the thesis – four are review articles and one is an original paper. The author has won a research grant sponsored by the Medical University Varna.

Some critical comments and recommendations

The readability of the manuscript is not good due to the multiple numbers and analysis variants in the text body. I would suggest that placing the number data in tables including a brief summary of the method and factors in the analysis where applicable. Furthermore, the numbers of the participants in the subgroups in the various results subsections are lacking. The reader is forced to calculate them himself and in some places the available data is not sufficient for the task.

Statistical methods: The statistical methods are described too briefly. Besides, the small sample size and the even smaller sizes of the subgroups might warrant the use of non-parametric approach. Moreover, the distribution of some of the data visibly deviated from normal. Some subgroups

comprise less than ten cases, even as few as three to four (e.g. control subjects with HDL<1.3 or LDL<2.6) that preclude reasonable summary statistics.

One weakness of the study that deserves mentioning is the lack of age adjustment in most of the analyses of the associations between the bone and the anthropometric and metabolic variables such as arterial hypertension. The age adjustment in the regression models might eliminate some correlations among typically age-dependent phenomena and characteristics and would improve the interpretation of the results.

Body weight, body mass index and waist circumference are associated and might complicate the interpretation of the results if included together into the equations due to co-linearity.

Some of the presented correlations are superfluous and might simplify the text if omitted. Some examples are the age and the duration of the menopause, DPDS/Cr and eGFR, eGFR and BW/BMI as well as correlations of both BMD and T-score with various variables. The correlation with BMD would be sufficient. The addition of the T-score does not add any value since BMD and the T-score are intrinsically associated. eGFR is inversely proportional to the creatinine excretion. The latter is the denominator in the DPD/Cr calculation. Finally, the age is a major factor in the FRAX equation and the correlation between age and the calculated Fx risks is meaningless. The separate analysis of each lumbar vertebra BMD/T-score is equally unnecessary.

A few words on the ROC analysis – The overlap of the data curve coincides with the diagonal reference line means that the tested variable does not contribute to the discrimination of the positive from the negative cases. In my opinion the results from the ROC curve analysis in the dissertation should be interpreted cautiously, e.g. "the measurement of CTX, DPD/Cr or OC cannot reliably help in the discrimination of the subjects with osteoporosis".

Despite the critical comments, the presented thesis on the associations of between bone health and some aspects of metabolism in postmenopausal women is interesting and complies with the formal requirements for a doctoral thesis. The candidate has demonstrated good knowledge of the topic and ability to explore the area, formulate a hypothesis, choose the proper methods, plan and execute the experiments and analyze and report the results. The confirmatory rather than pioneering character of the results of the dissertation is not a disadvantage. Moreover, they outline the directions for further research for Dr Radina Dimitrova, which I highly recommend.

In conclusion, it is my conviction that the dissertation with the presented published papers conforms fully to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

I recommend to the respected members of the Scientific Jury to give their positive vote and award Dr Radina Stoyanova Dimitrova the educational and scientific title Doctor of Philosophy.

08 February 20222 г.