

REVIEW

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Regarding:

Dissertation thesis for the acquisition of the educational and scientific degree "Doctor" (PhD) in professional field 7.1. "Medicine", scientific specialty "Pediatrics"

Author: Dr. Vilhelm Ruslanov Mladenov

Topic:

"Polycystic Ovary Syndrome in Adolescence – Modern Aspects and Guidelines for Diagnosis"

Scientific Supervisor: Prof. Violeta Mihova Iotova, MD, PhD, DSc

I. RELEVANCE OF THE PROBLEM

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic condition that has a significant impact on menstrual function, ovarian function, and a woman's reproductive potential.

However, the diagnosis of PCOS in adolescence poses a particular challenge. Menstrual irregularities, acne, changes in body weight, and relative hyperandrogenism can, to some extent, be part of normal pubertal development. Therefore, the application of diagnostic criteria used in adult women cannot always be directly translated to the adolescent population.

From a gynecological perspective, the early and correct identification of high-risk patients is of utmost importance. This allows for timely monitoring of menstrual and ovarian function, assessment of metabolic risk, and implementation of appropriate measures to prevent future reproductive and metabolic complications. In this context, the search for objective, reproducible, and sufficiently specific biomarkers to assist in diagnosis is fully justified. Focusing the present dissertation on AMH, FAI, androstenedione, and selected microRNAs as potential diagnostic tools is of particular interest.

Thus, the dissertation goes beyond the scope of the traditional clinical approach and explores opportunities to complement it with laboratory and molecular markers.

II. STRUCTURE AND VOLUME OF THE DISSERTATION

The dissertation thesis consists of 173 pages, includes 25 tables and 16 figures, and the references list contains 180 literary sources, the vast majority of which are in Latin script. The work is structured in a logical sequence, including an introduction, literature review, aim and objectives, material and methods, results, discussion, conclusions, scientific contributions, conclusion, and publications related to the dissertation.

III. REVIEW OF THE LITERATURE

The literature review is detailed and demonstrates thorough preparation of the PhD student regarding the issue under consideration. The main aspects of PCOS are examined – etiology and pathogenesis, clinical manifestations, hormonal disorders, metabolic abnormalities, and diagnostic criteria.

The examination of the issue of hyperandrogenism and menstrual irregularities in adolescence is extremely important, as these most frequently direct the clinician toward a PCOS diagnosis.

Substantial attention is devoted to AMH, FAI, and novel molecular markers. This imparts a modern character to the literature review and establishes a solid theoretical foundation for the author's own study.

IV. AIM AND OBJECTIVES

The aim of the dissertation thesis is clearly formulated and focused on improving and enriching the diagnostic toolkit in adolescent patients with PCOS.

The five set objectives are logically connected to the aim and encompass the clinical, hormonal, metabolic, and molecular characterization of the study population. I find the inclusion of an objective related to the study of selected miRNAs particularly appropriate. It lends originality to the dissertation research and places it within the context of contemporary trends in the search for novel biomarkers.

V. MATERIAL AND METHODS

The study is a case-control design and includes a total of 59 girls aged between 12.9 and 17.9 years:

- 36 patients with PCOS;
- 15 healthy controls;
- 8 patients with idiopathic hyperandrogenism.

From an OB/GYN standpoint, the inclusion of the idiopathic hyperandrogenism group is also relevant. This group allows one of the most important practical questions to be raised – whether a studied marker is specific to PCOS or rather reflects the presence of hyperandrogenism regardless of its etiology.

A comprehensive set of methods was utilized – clinical, anthropometric, hormonal, biochemical, ultrasound, and molecular-biological. Assessment of menstrual function, clinical hyperandrogenism via the modified Ferriman-Gallwey Score, pubertal

development according to Tanner, blood pressure, as well as carbohydrate and lipid metabolism was performed. Sex hormones, SHBG, AMH, and FAI were evaluated, along with selected miRNAs using qPCR. The statistical methodology is appropriately selected, with particular importance attached to the use of ROC analysis to evaluate the diagnostic value of the examined parameters.

VI. RESULTS AND THEIR CLINICAL INTERPRETATION

The obtained results are presented sequentially and subjected to appropriate statistical analysis.

1. Menstrual Function and Clinical Hyperandrogenism

One of the most important results is the identification of oligomenorrhea as the main clinical feature of patients with PCOS. This confirms the necessity for menstrual cycle irregularities in adolescents to be carefully evaluated, especially when combined with clinical or laboratory signs of hyperandrogenism.

Hirsutism is the leading clinical manifestation of hyperandrogenism in the studied group, with a significantly higher Ferriman-Gallwey Score being observed. Of interest is the established link between the degree of hirsutism and parameters of insulin resistance. This result has practical value, as it shows that clinical evaluation of hyperandrogenism should not be viewed in isolation from metabolic status.

2. Overweight and Metabolic Risk

Approximately two-thirds of patients with PCOS are overweight or obese. Of particular significance is the high frequency of insulin resistance and hyperinsulinemia. The observed disturbances in the lipid profile, as well as elevated blood pressure in a significant proportion of patients, confirm the multisystemic nature of PCOS.

These results are also of substantial importance for obstetrics and gynecology practice. A patient with PCOS should not be viewed solely through the prism of an irregular menstrual cycle or hyperandrogenism. As early as adolescence, it is necessary to assess her cardiometabolic risk as well.

3. Assessment of AMH, Androstenedione, and FAI

This is one of the most essential parts of the dissertation thesis. The obtained results demonstrate potential diagnostic value for several hormonal parameters.

ROC analysis determined the following cut-off values:

- Testosterone – 1.13 nmol/l;
- Androstenedione – 11.6 nmol/l;
- AMH – 23.6 pmol/l;
- FAI – 6.2.

Particularly impressive is the 100% specificity of androstenedione at the defined cut-off value, albeit with a sensitivity of 69%.

AMH shows good sensitivity – 86%, at a specificity of 65%.

FAI shows a sensitivity of 72% and a specificity of 90%.

From a gynecological perspective, it is precisely the combination of these parameters that is particularly interesting. Instead of relying on a single isolated marker, the results support the concept of a multiparametric diagnostic approach, in which clinical characteristics are complemented by hormonal and biochemical indicators. This represents one of the main scientific and practical outcomes of the dissertation.

4. MicroRNAs as Novel Diagnostic Biomarkers

The most original part of the dissertation thesis is the investigation of circulating microRNAs. The author examined six miRNAs and established diagnostically significant characteristics for miR-320-5p, miR-103a-5p, miR-15a-5p, and miR-194-5p. Particularly impressive are the results for miR-320-5p and miR-103a-5p, which demonstrated 100% specificity and 76% sensitivity in differentiating PCOS patients from healthy controls. miR-15a-5p showed 91% specificity and 76% sensitivity.

Of special practical importance is the investigation of the ability of these markers to differentiate PCOS from other hyperandrogenic conditions. This is precisely the clinically relevant question, because in real-world gynecological practice, the diagnostic challenge is not always distinguishing between a "sick" and a "healthy" patient, but between PCOS and other causes of hyperandrogenism. In this sense, the study of miRNA represents a major scientific contribution of the dissertation.

At the same time, I consider the author's cautious approach in interpreting these results to be correct. The obtained data are promising, but do not yet justify defining these molecules as established diagnostic criteria.

VII. DISCUSSION

The discussion is well structured, and the results are compared with data from contemporary literature. The author demonstrates the ability to critically interpret the findings. I positively evaluate the fact that the limitations of the diagnostic parameters and methods used were discussed, as well as the need for validation of the obtained results in larger populations.

From the perspective of an obstetrics and gynecology specialist, I would emphasize that the results should be viewed primarily as a step toward optimizing early diagnosis, rather than a proposal for an immediate change to established diagnostic criteria. This distinction is important and, in my opinion, correctly reflected in the dissertation thesis.

VIII. SCIENTIFIC CONTRIBUTIONS

I accept the contributions specified by the PhD candidate as real and arising from the conducted study.

Among them, I would highlight the following as particularly significant:

Scientific Contributions:

1. New data have been obtained regarding the clinical, hormonal, and metabolic profiles of adolescent patients with PCOS.
2. Potential diagnostic cut-off values for AMH, FAI, and androstenedione have been established.
3. For the first time in Bulgaria, the expression of selected circulating miRNAs was studied in adolescent girls with PCOS and control groups.
4. The potential diagnostic value of miR-320-5p, miR-103a-5p, miR-15a-5p, and miR-194-5p has been established.

Scientific and Applied Contributions:

5. The obtained results support the potential use of AMH, FAI, and androstenedione as complementary indicators in the diagnostic process.

6. Significant metabolic and cardiovascular abnormalities were established as early as adolescence, justifying the need for comprehensive monitoring of these patients.
7. A foundation has been created for future validation of novel molecular biomarkers in PCOS.

IX. PUBLICATION ACTIVITY

The results of the dissertation thesis have been presented in scientific publications and at scientific forums. Among them are publications dedicated to diagnostic criteria for PCOS in adolescence, hyperandrogenism, and the potential diagnostic utility of microRNAs.

Particularly significant is the publication:

Mladenov V., Radanova M., Galcheva S., Iotova V. Selected microRNAs as Potential Diagnostic Biomarkers in Polycystic Ovary Syndrome in Adolescent Girls. Applied Sciences. 2025;15:2772.

There is also a publication in *Scripta Scientifica Medica*, as well as a presentation of the results at the annual meeting of the European Society for Paediatric Endocrinology in Liverpool in 2024. The publication activity demonstrates that the findings of the dissertation have reached the scientific community and have been presented internationally.

X. BRIEF BIOGRAPHICAL DATA

Dr. Vilhelm Ruslanov Mladenov was born on February 6, 1970, in Varna. He graduated from First English Language School – Varna and completed his higher medical education at Medical University – Varna in 1995. He acquired a specialty in "Pediatrics" in 2002, and in 2007 – a specialty in "Pediatric Endocrinology and Metabolic Diseases".

His professional career includes working as an expert at the Regional Health Insurance Fund (RHIF) – Varna and as a pediatrician at "St. Anna" University Hospital – Varna. Since 2009, he has been a physician in the First Pediatric Clinic at "St. Marina" University Hospital – Varna, working as a pediatric endocrinologist. Since 2017, he has held the academic position of "Assistant Professor" in the Department of Pediatrics at Medical University – Varna.

His professional qualifications are complemented by numerous specialized trainings in the field of pediatric endocrinology and rare diseases. He completed trainings and fellowships within ESPE and ISPAD, including a fellowship in Ghent, Belgium, in the field of Disorders of Sex Development (DSD). He holds professional certificates and practical skills in endocrine ultrasound, DXA scanning for bone and fat density, insulin pump therapy, and continuous glucose monitoring (CGM) systems.

Dr. Mladenov is actively engaged in research focused on problems in pediatrics and pediatric endocrinology. He is author and co-author of over 30 scientific publications in Bulgarian and international scientific journals, with over 200 citations. A significant part of his research interests and publication activity is related to rare diseases. He has participated as an invited speaker in national and international scientific forums.

He is a member of several national and international professional organizations, including the Bulgarian Society of Endocrinology, European Society for Paediatric Endocrinology (ESPE), International Society for Pediatric and Adolescent Diabetes (ISPAD), Bulgarian National Association of Pediatric Endocrinology, Bulgarian Society of Human Genetics and Genomics, and the Bulgarian Medical Association.

XI. GENERAL EVALUATION OF THE DISSERTATION

The dissertation thesis of Dr. Vilhelm Ruslanov Mladenov represents a complete scientific study with clearly expressed relevance, originality, and practical significance. As an obstetrics and gynecology specialist, I would identify its interdisciplinary character as a particularly strong aspect of the dissertation.

Adolescent PCOS is viewed not merely as an endocrine disorder, but as a condition that, even in the early years of reproductive life, can be associated with menstrual irregularities, hyperandrogenism, metabolic changes, and elevated cardiometabolic risk.

I particularly appreciate the candidate's effort to seek objective diagnostic markers capable of complementing clinical evaluation. The findings regarding AMH, FAI, and androstenedione are clinically interesting, and the investigation of miRNA undoubtedly constitutes the most original part of the dissertation.

The scientific value of the work is further enhanced by the fact that the author does not overestimate the results. On the contrary – the limitations of the study are acknowledged, and the need for larger, multicenter studies is emphasized. This approach demonstrates the scientific maturity and critical self-evaluation required of a PhD candidate.

XII. CONCLUSION

Based on the analysis performed on the presented dissertation thesis, I consider the work of Dr. Vilhelm Ruslanov Mladenov titled: *"Polycystic Ovary Syndrome in Adolescence – Modern Aspects and Guidelines for Diagnosis"* to be an original, relevant, and scientifically sound study containing substantial scientific and practical contributions.

The dissertation thesis demonstrates good theoretical background, skills in planning and conducting scientific research, experience with modern laboratory and molecular-biological methods, adequate statistical processing, and the ability to critically interpret results. The data obtained have potential value for refining the diagnostic approach to adolescent PCOS, and the study of circulating miRNAs opens a promising direction for subsequent research.

I consider that the dissertation thesis meets all requirements for the acquisition of the educational and scientific degree "Doctor" (PhD).

Based on all the above, I give my positive evaluation of the dissertation thesis and confidently recommend to the esteemed Scientific Jury to award Dr. Vilhelm Ruslanov Mladenov the educational and scientific degree "Doctor" (PhD) in the scientific specialty "Pediatrics", professional field 7.1. "Medicine".

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REVIEWER:

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